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Nitrogen Accumulation by Rice.

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NITROGEN ACCUMULATION BY RICE

A Dissertation

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in

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by
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As you read through this dissertation, you might conclude that I was a bit busy during my sojourn in Louisiana. I was, but I was not alone. And because I was not alone, I am able to submit this dissertation. Many individuals contributed to my research, and I am indebted to them, and offer my gratitude. I cannot possibly name all the individuals, but I do wish to mention a few. Dr. John Kovar and Dr. Patrick Bollich served as my major professors, and helped me throughout the project. The quantity and quality of their help is phenomenal. Dr. Charles Lindau, Dr. Raul Macchiavelli, and Dr. Mary Musgrave served on my committee, and they always had time for my needs. Dr. Michael Braverman was kind enough to represent the Graduate College on my committee. Ms. Jessica Guidry, Mr. William Leonards, Mr. Greg Meche, Mr. Ronald Reagan, and Mr. Gerry Romero took care of my crop and all my samples at the Rice Station. A special word of thanks to the many student workers at the Rice Station and the Root Lab. Because of all this help, I was able to finish this dissertation, and even find a little time to hike and play racquetball, build furniture and play music, as well as some noble pursuits that my left hand has not told my right hand about. Thanks to everyone.

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ABSTRACT

Consumption of rice is worldwide, and the rice-eating population is expanding rapidly. Breeding high yielding varieties has helped increase rice production. However, effective management practices have also been useful, and will probably become more vital as breeding efforts approach the genetic limits of the rice plant. Nitrogen (N) management is particularly important in reducing N losses and increasing grain yield. The objectives of this study were (1) to determine the amount of N accumulation by rice at different stages of plant development, (2) to determine if differences in N accumulation exist between rice varieties, and (3) to determine if differences in N accumulation exist between different N sources. For each objective, N accumulation as well as the associated rice plant growth was of interest.

Field studies were conducted at the Rice Research Station in Crowley, LA, in 1993, 1994, and 1995. Various varieties of rice were planted in both drill-seeded and water-seeded culture. Urea and ammonium sulfate were used as N sources. Microplots and ^{15}N were utilized. Nitrogen accumulation, straw, grain, and total dry matter, and root length density were measured.

Nitrogen accumulation occurred throughout the growing season, while dry matter increased after panicle differentiation. Grain yields were constant from season to season, but straw yield, root growth and N accumulation varied because of air temperature. Responses were the same in drill-seeded and water-seeded culture. Most midseason N was accumulated by rice plants within 7 days after topdress. There were

no differences in N accumulation among rice varieties, although grain yields differed. Modern varieties produced more grain than older varieties, and among modern varieties, semi-dwarf varieties produced more grain than tall varieties. Ammonium sulfate and urea were equally effective sources of N for rice, and S did not appear to affect those results. Microplot size and presence of a retainer were found to have an effect on experimental results. A 30 x 30 cm microplot without a retainer was found to be the best substitute for normal field conditions.

INTRODUCTION

Rice (*Oryza sativa* L. and *Oryza glaberrima* Steud.) is grown throughout the world, and has been grown in the Americas ever since Columbus discovered that the New World had already been discovered. Consumption of rice is worldwide, and the rice-eating population is expanding rapidly. Rice production has been able to keep pace with these increases, and will need to continue to do so in the future. Breeding high yielding varieties has helped worldwide rice yield increases. Effective management practices have also been useful, and will probably become more vital as breeding efforts approach the genetic limits of the rice plant. Careful nitrogen (N) management has reduced N loss in the field, and has been effective in increasing the yield of modern rice varieties. Further research is needed to introduce newer N management strategies that will provide more efficient and effective N use.

The present study was initiated to learn more about N accumulation by rice. A knowledge of the amount and time of N accumulation is useful for developing management strategies in the field. Furthermore, research in the field provides data from production-like conditions that more readily point to farm-level management strategies.

The general objectives of this study were (1) to determine the amount of N accumulation by rice at different stages of plant development, (2) to compare N accumulation in drill-seeded (DS) and water-seeded (WS) culture, (3) to determine if differences in N accumulation exist between rice varieties, and (4) to determine if

differences in N accumulation exist between different N sources. For each objective, N accumulation as well as the associated rice plant growth was of interest.

The first two objectives were accomplished with two experiments. The first experiment considered plant growth and N accumulation throughout the season from an early season N application in both DS and WS. The second experiment considered plant growth and N accumulation from a midseason N application. The third objective was accomplished with an experiment comparing 18 rice varieties at three N levels. The fourth objective was accomplished with an experiment comparing plant response to ammonium sulfate and urea. Because many of these experiments involved ^{15}N and microplots, a final experiment was conducted to determine the effect of that growing environment on plant growth and N accumulation.

LITERATURE REVIEW

Rice is one of the major food crops in the world today (Fageria *et al.*, 1991), estimated to be the primary source of calories for 40% of the world's population (De Datta 1981). *O. sativa* is of South and Southeast Asian origin, and is the predominant species grown throughout the world, while *O. glaberrima* is of African origin and is limited to ever decreasing cultivation in Africa (Efferson, 1994; Fageria *et al.*, 1991; Mikkelsen and De Datta, 1991). *O. sativa* is generally grown under flooded (lowland) conditions, while *O. glaberrima* is generally grown under drained (upland) conditions (Efferson, 1994). There are three races of *O. sativa*. The indica race is tropical, the javanica race is also tropical but has spread to some sub-tropical areas, and the japonica race is sub-tropical and temperate (Mikkelsen and De Datta, 1991). Rice production today is predominantly in Asia, although it is grown and consumed in all continents except Antarctica (Fageria *et al.*, 1991).

Rice in North America had numerous failed introductions, beginning with the second voyage of Columbus in 1493 (Efferson, 1994). A damaged ship sailing into the port of Charleston, South Carolina, in 1694 brought the first rice seed that was successfully planted ('Carolina Gold'), and a rice industry developed in South Carolina and adjacent states. Rice was first introduced into Louisiana by Bienville in 1718, and large scale mechanized culture in Southwest Louisiana was started by a visiting Iowa wheat farmer in 1884 (Bagent *et al.*, 1987; Efferson, 1994).

During the past 30 years, the use of high yielding rice varieties has spread (Chang and Li, 1991). These varieties have a greater demand for inputs than did their parent lines, and N is high on the list of required inputs (Chang and Li, 1991). Although the price of N fertilizer is low in comparison to the price of other inputs and the value of the crop (at least in the wealthier industrialized nations), it is not an insignificant amount. Furthermore, the cost of unnecessary N applications cannot be justified economically, environmentally, and in lost yield. For these reasons, there has been much recent interest in the various aspects of N management in rice crop production.

Rice Plant Growth and Development

Growth of the rice plant can be divided into three phases of development, (1) the vegetative phase, from germination to panicle initiation (PI), (2) the reproductive phase, from PI to flowering (50% floral pollination) or heading, and (3) the ripening phase, from flowering to maturity (De Datta, 1981; Stansel, 1975; Vergara, 1991). These phases affect the three components of yield, (1) the number of panicles per unit land area, (2) the average number and size of spikelets per panicle, and (3) the average weight of individual grains, respectively (De Datta, 1981; Stansel, 1975). In standard long-season varieties (about 150 days to maturity), the vegetative phase ends with a lag period during which maximum tillering, internode elongation, and PI occur in succession. In modern short-season varieties (105-120 days to maturity), maximum tiller number, internode elongation, and PI occur at about the same time. The length of the lag period is responsible for differences in days to maturity in different varieties

grown in the same climatic and geographic region, since the vegetative phase up to maximum tillering, and the reproductive and ripening phases are approximately equal in length in all varieties (De Datta, 1981; Vergara, 1991).

Fertility at each phase of development affects the growth of the plant and the associated component of yield (Stansel, 1975). Murata (1969) reported that there is a close correlation between the number of spikelets per panicle and the total N absorbed up to flowering. Nitrogen accumulation from PI to flowering increases carbohydrate accumulation both at heading and at maturity, thereby increasing the grain yield. De Datta (1986) reported that rice plants demand N at early- and mid-tillering to maximize panicle number, and at reproductive and ripening phases to maximize spikelets per panicle and percentage filled spikelets.

The rice crop depends on N available in the soil. Native soil N is frequently supplemented with additions of manure, green manure, or fertilizer N. Fertilizer N is generally applied in an available form, whereas manure and green manure have only a portion of the N in an available form. Regardless of the source, all N in the soil is subject to changes that simultaneously contribute to N availability and N loss.

Nitrogen Transformations in Soil

Nitrogen is added in greater amounts than any other nutrient to soils in the United States, and its relatively low cost has encouraged that practice. However, the N added is not necessarily used by the targeted crop, and furthermore, much of what the crop uses comes from sources other than that which is added as fertilizer. Nitrogen can go through a variety of transformations in soil, and this has been thoroughly reviewed

by numerous authors (Focht and Verstraete, 1977; Freney and Simpson, 1983; Haynes *et al.*, 1986; Stevenson, 1982). Transformations in flooded soils are very similar, although their special conditions alter the rate at which changes occur (Reddy and Patrick, 1986). Keeney and Sahrawat (1986), Mikkelsen (1987), Reddy and Patrick (1983), and Savant and De Datta (1982) have reviewed the literature specific to N transformations in flooded soils. Nitrogen transformations in both drained and flooded soils are summarized below.

Mineralization and Immobilization

Nitrogen mineralization (or ammonification) is the chemical transformation of N from organic forms into the ammonium (NH_4^+) or ammonia (NH_3). Nitrogen immobilization is the biological transformation of the inorganic forms of N (NH_4^+, NH_3, nitrite (NO_2^-), and nitrate (NO_3^-)) into organic forms (Jansson and Persson, 1982; Keeney and Sahrawat, 1986; Reddy and Patrick, 1983; Savant and De Datta, 1982). Both mineralization and immobilization occur simultaneously and interactively.

Photosynthesizing plants trap energy from the sun in the bonds of compounds which form their tissues (Jansson and Persson, 1982). When these plants die or their tissues are sloughed off, they decompose in the soil by the action of heterotrophic soil organisms that use their N-containing organic substances as sources of carbon and energy for themselves. Through this decomposition process, the organic forms of N are transformed into inorganic forms (mineralization), as are other elements, which are then available as nutrients for other photosynthesizing plants. Carbon, however, is given off as CO_2 during respiration, with some remaining in the soil solution (Haynes,

1986b). While plants are assimilating the inorganic products of mineralization, soil organisms use some of these products (including some of the carbon) as constituents of their cells and tissues (immobilization). However, these organisms (both heterotrophic and autotrophic) use very little of the products of mineralization. Aside from fertilizer additions, it is the net effect of the concurrent processes of mineralization and immobilization that determines the inorganic-N available in the soil environment. Nevertheless, mineralization is favored, as this is the major energy supplying activity of heterotrophs. Immobilization is a minor activity of the hetertrophs, occurring merely to sustain their tissues. However, when a residue with a high C:N ratio is present, immobilization is favored.

There are numerous factors which affect the mineralization of organic materials by heterotrophic soil organisms. Essentially, what affects soil organisms will affect mineralization. The chemical composition of the decomposing material, soil pH, moisture, temperature, and cultivation all affect mineralization.

The chemical composition of the decomposing material is a very important factor in the rate of mineralization (Haynes, 1986b). The lower the C:N ratio (signifying a relatively high N content compared to carbon content), the higher the rate of N mineralization. This guarantees that mineralization will be greater than immobilization. However, it is not easy to determine a critical C:N ratio, that is, a ratio above which immobilization will equal or exceed mineralization. This is partly because the E:N ratio (energy:N) is the important parameter which is approximated by the C:N ratio (Jansson and Persson, 1982). The E:N ratio is the ratio of the energy supplied by

mineralization of the N source to the N mineralized. Organic materials dominated by fungi in acidic soils need higher critical E:N ratios than those dominated by bacteria. It appears that this critical ratio differs depending upon the decomposing material, and with time for a given material. The initial lignin content of the organic material appears to have a greater effect on the rate of decomposition than the C:N ratio (Haynes, 1986b). A higher lignin content results in slower mineralization. Furthermore, with a higher lignin content, the initial N content has less influence. Likewise, a higher polyphenol content in the organic material also lowers the rate of mineralization. Aside from their structural inhibition to decomposition, polyphenols are known to inhibit fungal activity.

Soil pH is one of the major limiting factors in mineralization (Haynes, 1986b). Higher pH favors decomposition, although some mineralization will occur under all pH conditions, mediated by microorganisms suited to those conditions. Raising the pH of a site to neutrality has been shown to increase decomposition and subsequent N mineralization. The initial soil pH of flooded soils has an effect on mineralization during the early period of submergence (Savant and De Datta, 1982). Liming increases the availability of N in flooded soils (Keeney and Sahrawat, 1986) even though the pH approaches neutrality without lime.

Another factor affecting mineralization is moisture content (Haynes, 1986b; Savant and De Datta, 1982). When under considerable moisture stress, microbial growth is inhibited, although this inhibition is not manifested until potentials reach below -1000 to -5000 kPa. At higher soil moisture levels, a lack of oxygen reduces the

rate of biological activity (except for anaerobic bacteria, but these are less efficient) and reduces the rate of mineralization. For this reason, poorly drained and flooded soils generally maintain a high organic matter content. Drying and rewetting increases decomposition, primarily by causing flushes in N mineralization at wetting. This results from breaking soil aggregates apart and exposing organic material previously inaccessible to heterotrophs.

Higher temperature is associated with a higher rate of organic matter decomposition (Haynes, 1986b). Optimal temperature for decomposition is in the range of 45 to 60°C, with a lower temperature limit of about 0°C. However, for high temperature to be effective in mineralization, it must be accompanied by adequate moisture. This combination is more important than either factor by itself. It is generally believed that a fluctuating temperature does not favor microbial growth although some contrary data have been presented. Freezing and thawing, however, still seem to be an avenue for increased mineralization by exposing otherwise inaccessible organic material. In flooded soils, temperature is the most important factor controlling mineralization (Savant and De Datta, 1982).

Cultivation, as is common in agricultural soils, results in a rapid increase in decomposition and N mineralization (Haynes, 1986b). Since cultivation kills living vegetation, incorporates it into the soil profile, breaks up existing soil aggregates, and increases aeration (at least temporarily), it favors mineralization. This is true when other conditions (mentioned above), especially moisture content, are also favorable. However, the effect of cultivation is less clear in flooded soils because it affects N

utilization in others ways than by increasing mineralization (Keeney and Sahrawat, 1986). Soils with higher clay content tend to have higher soil organic matter content, suggesting that clay content negatively influences mineralization (Haynes, 1986b). However, the mechanisms for this process are not clear.

Nitrification

Nitrification is the process by which NH_4^+ in the soil is oxidized into nitrite and nitrate (Haynes, 1986a; Reddy and Patrick, 1983; Schmidt, 1982). Lack of oxygen generally inhibits nitrification in flooded soils (Keeney and Sahrawat, 1986). Therefore, nitrification occurs in the oxidized surface layer of flooded soils and the oxidized rhizosphere of wetland plants (Reddy and Patrick, 1986; Savant and De Datta, 1982). It is generally accepted that nitrification is mediated by autotrophic bacteria of the family *Nitrobacteraceae*, although there have been suggestions that there are other pathways in soil (Haynes, 1986a). In the atmosphere, nitric oxide near discharging lightning can be converted photochemically into NO_3^-, and then washed into the soil with precipitation. However this accounts for a very small part of the NO_3^- in the soil.

Under the *Nitrobacteraceae*-pathway, the reaction proceeds as follows (Focht and Verstraete, 1977; Haynes, 1986a; Reddy and Patrick, 1983; Schmidt, 1982):

$$\begin{array}{ccccccc} (-3) & & (0) & & (+1) & (+3) & (+5) \\ NH_4^+ & \rightarrow & NH_2OH & \rightarrow & (NOH) & \rightarrow NO_2^- \rightarrow & NO_3^- \\ & & & & \downarrow & & \\ & & & & N_2O & & \\ & & & & (+1) & & \end{array}$$

Nitrosospira briensis and *Nitrosomonas europaea* appear to be the dominant agents for the oxidation of NH_4^+ into NO_2^-, the latter being the most common in agricultural soils

amended with sewage sludge or manure. *Nitrosolobus multiformis* is also present, but in lower numbers. All three are obligate autotrophs. The oxidation of NO_2^- into NO_3^- is aided by *Nitrobacter winogradskyi*, which might be a facultative heterotroph. This reaction occurs rapidly, so there is no accumulation of NO_2^- in the soil. Chemical dismutation, the conversion of NOH to N_2O, occurs under anaerobic conditions.

Aside from the presence of appropriate bacteria, certain conditions must be maintained for the bacteria to oxidize N compounds (Schmidt, 1982). Being autotrophic, their carbon source is CO_2, although they derive their energy from the oxidation process. In addition, there must be an adequate supply of NH_4^+ for oxidation to occur. However, plant roots, mycorrhizal fungi, and heterotrophic microorganisms compete with the N-oxidizing bacteria for the available NH_4^+. Furthermore, when carbon-containing plant materials are sloughed off in the soil, NH_4^+ is rapidly immobilized in the newly formed microbial biomass, also decreasing the available NH_4^+. Because of these processes in some soils, especially in many grassland and forest ecosystems, there is little NH_4^+ available for nitrification regardless of the *Nitrobacteraceae* population. Therefore, little NO_3^- is produced for plant accumulation.

Autotrophic nitrifiers depend on NH_4^+ as an energy source (Haynes, 1986a; Schmidt, 1982). Therefore, reduced nitrification results from low concentrations of NH_4^+. However, high concentrations of NH_4^+ can also have a detrimental effect. There are varied reasons for this. First, there are toxic levels of NH_3 (pk_a 9.0) at high pH, and these are more dramatic in soils with low CEC. Second, there is an increase in salt

content of the soil. Third, there is a decrease in pH in ammonium sulfate ($(NH_4)_2SO_4$)-amended soils. It appears that the NO_2^--oxidizing bacteria are more sensitive to high NH_4^+ concentrations, sometimes resulting in an accumulation of NO_2^-. The ideal NH_4^+ concentration in soils for nitrification appears to lie between 400 and 800 $\mu g\ N\ g^{-1}$ soil. A high concentration of NO_3^- (the end product of the oxidation process) also represses further nitrification, although this is not common in the field.

Soil pH is one of the major limiting factors for nitrification (Haynes, 1986a; Savant and De Datta, 1982; Schmidt, 1982). Nitrifying bacteria perform well between pH 6 and 9 (best between 6 and 7), although the possibility of NH_3 toxicity increases with increasing pH, causing a decrease in NO_2^- oxidation and therefore NO_2^- accumulation. Below pH 4.5, the nitrifying bacteria are inhibited. Aluminum toxicity might be the primary reason for the inactivity of the autotrophs in acid soils. Despite the pH of a soil, a microsite might have a more favorable pH, and therefore, nitrification will occur while the surrounding conditions seem to indicate otherwise.

Aeration and moisture are reciprocal to one another in soil (Haynes, 1986a). At soil moisture potentials between -10 and -33 kPa (varying with soil physical properties), nitrification is maximized. Outside of these limits, the process retards. Nitrification occurs only in the oxidized layer of flooded soils and in the oxidized rhizosphere of wetland plants. Oxygen deficiency prevents nitrification in wet soils, but the mechanism is less clear in dry soils. Desiccation probably reduces the nitrifying population.

Nitrification is affected by temperature, and is favored most by temperatures between 25 and 35°C (Haynes, 1986a; Schmidt, 1982). However, localized strains of bacteria stretch these limits in many instances, with reports of optimal temperatures as low as 20°C and as high as 60°C. Flushes of nitrification have been reported to occur after fluctuating temperatures, much like the flush of nitrification seen after rewetting of a dried soil. As temperature increases, the solubility of O_2 in water decreases, and the demand for O_2 by heterotrophs increases.

There is some evidence that other factors affect nitrification in soils (Haynes, 1986a; Schmidt, 1982). The presence of oxidizable organic matter reduces nitrification because it increases the heterotrophic O_2 demand. This reduces the oxygen available to the nitrifying autotrophs. In many cases, however, nitrification proceeds normally in spite of this, if other environmental factors are favorable. Allelopathic effects of some plants might inhibit nitrification, causing an increase in the NH_4^+ concentration in the soil. A phosphorus deficiency can limit nitrification. High concentrations of trace elements, arising from amendments such as sewage sludge and industrial wastes, might inhibit nitrification. Finally, nitrifying bacteria appear to be sensitive to pesticides, especially soil fumigants and herbicides.

Pathways other than the autotrophic *Nitrobacteraceae* mediated one have been proposed (Haynes, 1986a; Schmidt, 1982). One suggestion is that nitrification is mediated by heterotrophs, mainly fungi. These heterotrophs can operate at lower pH, with an optimum around 4.5, and at a higher temperatures (50-60°C). This would explain some observed nitrification under conditions in which the autotrophs are not

known to function. Another proposed pathway is through methane-oxidizing bacteria, which are found at aerobic sites bordering anaerobic sites. These bacteria use CH_4, CH_3OH, and CH_3OCH_3 as their carbon sources, but high concentrations of NH_4^+ will inhibit the oxidation of CH_4. A final proposed pathway suggests that NO_3^- formation results from chemical oxidation. This has been demonstrated in very acid soils (below 4.5) with NO_2^- serving as both an electron donor and acceptor, or mediated by manganese oxides.

Denitrification

Denitrification is the microbial reduction of NO_2^- or NO_3^- to gaseous N (mostly N_2 and N_2O) (Fillery, 1983; Firestone, 1982; Haynes and Sherlock, 1986). The generally accepted pathway of denitrification is thought to be (Firestone, 1982):

$$\begin{matrix} (+5) & (+3) & (+2) & (+1) & (0) \\ NO_3^- & \rightarrow NO_2^- & \rightarrow (NO) & \rightarrow N_2O & \rightarrow N_2 \end{matrix}$$

These reactions are mediated by certain aerobic bacteria (mostly chemoheterotrophs) which are able to respire in the absence of O_2 by reducing NO_3^--N or NO_2^--N into the gaseous forms. The N_2O and N_2 formed in the reaction are released into the atmosphere and are not assimilated by plants. Dinitrogen is the major product of denitrification (Keeney and Sahrawat, 1986). It is not known exactly which bacteria mediate the reaction, because of the presence in soil of about 23 different genera which have the capability of using N-oxides as electron acceptors in place of O_2 (Focht and Verstraete, 1977). Those most commonly studied are *Paracoccus denitrificans*, *Pseudomonas denitrificans*, and *Pseudomonas perfectomarinus*. Some N-fixing

organisms are also known to have the ability to denitrify under anaerobic conditions, particularly some species of *Rhizobium*.

Since most denitrifiers are heterotrophs, a good supply of readily decomposable organic matter increases the rate of denitrification (Fillery, 1983; Firestone, 1982). This occurs for two reasons, 1) organic matter contributes electrons and is a source of cellular material for the heterotrophs, and 2) since the presence of organic matter increases other heterotrophic activities, such as mineralization, then microbial respiration is increased, causing rapid depletion of the available oxygen, which creates the anaerobic conditions required for denitrification. Aside from the organic matter already in a soil, it has been found that amendments, such as plant residues or manure, increase denitrification rates, provided they are readily decomposable. Water-soluble organic substances with low molecular weight have a much greater effect on denitrification than high molecular weight substances with low water solubility (Savant and De Datta, 1982).

For denitrification to occur, there must be ample NO_2^- or NO_3^- in the soil (Fillery, 1983; Firestone, 1982; Focht and Verstraete, 1977). If other conditions are ideal for denitrification to occur, higher NO_2^- or NO_3^- concentration increases the rate of denitrification (Firestone, 1982). However, at very high NO_3^- concentration, denitrification becomes independent of NO_3^- concentration. Also, at very high NO_3^- concentration, the NO_3^- can inhibit the reduction of N_2O into N_2, increasing the relative amount of N_2O. This latter effect increases with a decrease in soil pH. In addition,

NO_3^- produced in the oxidized layer of a flooded soil can diffuse into the aerobic layer below, and provide the substrate for denitrification (Savant and De Datta, 1982).

Denitrification increases with an increase in soil pH, since the denitrifying bacteria are sensitive to low pH (Firestone, 1982; Focht and Verstraete, 1977). In addition, at higher pH, there might be a temporary increase in the solubility of soil organic matter. The optimum pH has been identified as 7-8, although between 6 and 8 there appears to be little positive response to pH. Below pH 6, denitrification is inhibited, and bacterial activity is very slow around pH 4. This could be because of toxic levels of soil aluminum and manganese, although even at these pH levels there is still some denitrification, possibly because of microsites with a more favorable pH.

Aeration and moisture have enormous impact on denitrification (Fillery, 1983; Firestone, 1982; Focht and Verstraete, 1977). Denitrification only occurs under anaerobic conditions. Therefore, well structured and aerated soils do not support denitrification, although denitrification can still occur if the localized microsite in a soil aggregate is anaerobic. Furthermore, because O_2 diffuses more slowly through water than air, microsites which are low in oxygen but are not waterlogged fail to receive more oxygen because of the slow rate of diffusion through the surrounding waterlogged soil. As the soil moisture content increases and the available oxygen decreases, denitrification also increases. When N_2O is produced in a well-aerated soil, it is likely that it is produced as a by-product of nitrification, although the mechanisms for this are not well understood.

Between 10 and 35°C, the rate of denitrification is very temperature dependent, increasing by a factor of 2 for every 10°C (Fillery, 1983; Firestone, 1982; Focht and Verstraete, 1977). The optimum temperature is about 65°C. Below 10°C and above 75°C denitrification is slow. However, above 50°C there might be less biological denitrification and more chemical decomposition. Local strains of bacteria might also have other optimal limits from those quoted above. Related to temperature, it appears that more denitrification occurs in the afternoon than at night, and more during the summer than in winter. Aside from the quantity of denitrification related to temperature, it has been shown that an increase in temperature favors N_2 over N_2O, although in some cases temperature has less of an effect. In addition, temperature has a marked effect on O_2 solubility, diffusion and consumption. Decreasing temperature increases O_2 solubility and decreases O_2 consumption, both creating a more aerobic environment, although diffusion will slow.

Other factors have at least a marginal impact on denitrification (Firestone, 1982). The presence of plant roots, contributing carbon-containing compounds to the rhizosphere, help nourish denitrifiers (as well as other heterotrophs) and create microsites deficient in O_2. Both aid denitrification. At the same time, evapotranspiration by plants can cause drier soil near the roots, thus increasing the rate of O_2 diffusion to this area, and decreasing denitrification. Furthermore, plant uptake of NO_3^- reduces the quantity of substrate for denitrification. Certain plants, such as rice, pump oxygen into the rhizosphere through aerenchyma, forming an oxidized area around the roots. The net effect of these conditions could vary greatly depending on

other factors. Under reduced or zero tillage, there is less aeration and higher moisture levels at the surface, which favor denitrification. Potential for denitrification decreases with increased depth in the soil profile. The effect of depth is related mostly to the size of the population of denitrifiers at the different depths.

Ammonia volatilization

The process by which gaseous NH_3 is lost from the soil surface to the atmosphere is called ammonia volatilization (Freney *et al.*, 1983; Haynes and Sherlock, 1986). Soil adsorbed NH_4^+ is converted into gaseous NH_3 by the following pathway:

$$NH_4^+{}_{(ad)} \rightleftharpoons NH_4^+{}_{(aq\ in\ soil)} \rightleftharpoons NH_{3(aq\ in\ soil)} \rightleftharpoons NH_{3(g\ in\ soil)} \rightleftharpoons NH_{3(g\ in\ atm)}$$

In addition to adsorbed NH_4^+, other sources for ammonia volatilization can be NH_4^+ from soil organic matter or plant residues, NH_4^+ or urea from fertilizer or organic amendments, anhydrous ammonia fertilizers, or NH_4^+ from mineralization reactions.

Under certain conditions, ammonia volatilization is a spontaneous process which occurs without the aid of microorganisms. The primary determinant as to whether gaseous NH_3 will remain in the soil solution or be volatilized is the difference in partial pressure between NH_3 in the air and NH_3 dissolved in water (Freney *et al.*, 1983; Nelson, 1982). Since atmospheric NH_3 gas concentration is generally low, they do not limit volatilization. Rather, a small amount of gaseous NH_3 in the soil surface is likely to be greater than the atmospheric concentration, and result in volatilization. The extent of the loss will be determined by the concentration of gaseous NH_3 in the soil solution. Even though NH_3 is the most soluble gas known, suggesting that

waterlogged soils would protect NH_3 from volatilization by storing the gas in solution, the phenomenon described above applies equally to well aerated and flooded soils.

In flooded soils, floodwater and atmospheric conditions affect ammonia volatilization more than soil parameters (Savant and De Datta, 1982). Soil parameters are more important in drained soils.

There is a positive relationship between the rate of ammonia volatilization and the amount of NH_4^+ added to a soil (Haynes and Sherlock, 1986; Nelson, 1982). In addition, factors which affect NH_4^+ concentration in the soil solution have the same effect as increasing NH_4^+-containing amendments. Some of these factors are plant uptake, nitrification, denitrification, leaching, immobilization, and NH_4^+ fixation (all leading to a decrease in NH_4^+ concentration in the soil solution and a decrease in NH_3 loss). In addition to the amount of amendments to the soil and the soil conditions, the form in which N is added and the method of application can affect ammonia volatilization. Application of anhydrous ammonia can result in great N losses, except when it is injected, in which case there are minimal losses. Surface application of alkaline fertilizers such as NH_4OH or urea usually lead to greater ammonia volatilization than application of neutral or acidic fertilizers such as $(NH_4)_2HPO_4$ or ammonium sulfate. By either incorporating the fertilizer or by adding neutral ammonium salts or acidifying agents, one can reduce NH_3 loss.

The equilibrium between NH_4^+ and NH_3 can be represented in two different ways (Haynes and Sherlock, 1986):

a) $$NH_4^+ + OH^- \rightleftharpoons NH_4OH \rightleftharpoons NH_3 + H_2O$$

b) $NH_4^+ \rightarrow NH_3 + H^+$

$H^+ + OH^- \rightarrow H_2O$

As the soil pH increases (hydroxide ion concentration increases), the reaction is driven to the right, resulting in an increase in NH_3 production (pathway a) (Haynes and Sherlock, 1986). However, if pathway (b) is considered, one can conclude that, as volatilization occurs, the pH decreases (Haynes and Sherlock, 1986). Therefore, the initial pH has an effect on volatilization, where a high initial pH favors volatilization, and as volatilization continues, the process creates pH conditions which are less favorable to continued volatilization. Since much volatilization occurs in some very acid soils, conditions other than soil pH influence the process. However, NH_3 loss generally increases with increasing pH in most other soils (Freney *et al.*, 1983; Nelson, 1982), as alkaline pH favors aqueous NH_3 whereas acidic or neutral pH favors NH_4^+ (Reddy and Patrick, 1983). Furthermore, the pH of floodwater is considered the single most important determinant of ammonia volatilization (Keeney and Sahrawat, 1986).

Although research results are frequently contradictory, it appears that greatest ammonia volatilization occurs when soils of high moisture content dry (Haynes and Sherlock, 1986; Nelson, 1982). This is because NH_3 concentration in the soil solution increases as drying proceeds, resulting in greater losses than if the soil had not dried. Furthermore, as drying proceeds, water moves upward in the soil profile, and this is accompanied by movement of NH_4^+ and NH_3 to the soil surface. Losses of NH_3 in an air-dry state are minimal.

With higher temperature up to about 45°C, there is a greater proportion of aqueous NH_3 and gaseous NH_3 present in the soil solution; hence, there is an increase in the likelihood of NH_3 loss (Freney *et al.*, 1983; Haynes and Sherlock, 1986; Nelson, 1982). This is especially true after application of NH_4^+ or NH_4^+-producing amendments, since the concentration of the reactants for the volatilization reaction are increased. The pattern of NH_3 loss follows both a daily and a seasonal cycle, with losses greatest when temperature is highest.

Other factors also affect ammonia volatilization. The rate of airflow over the surface has been found to directly affect ammonia volatilization (Freney *et al.*, 1983; Haynes and Sherlock, 1986). This is especially true for flooded soils. An increase in wind speed removes NH_3 from the atmosphere just above the soil or floodwater surface. This reduces the NH_3 concentration in the air and favors further volatilization. Additionally, when the relative humidity of air flowing over the soil or floodwater surface is higher, ammonia volatilization is higher (Nelson, 1982). Soils with a high CEC, such as fine-textured soils, are more likely to adsorb NH_4^+ (Freney *et al.*, 1983; Haynes and Sherlock, 1986; Nelson, 1982). This reduces NH_3 production and volatilization. Calcareous soils are susceptible to volatilization. This is because of the effect of the $CaCO_3$ on the pH and buffering capacity of the soil, and because some anions from applied fertilizers form insoluble precipitates with calcium (such as F^-, SO_4^{-2}, HPO_4^{-2}). Both result in easier volatilization of the remaining NH_4^+ than if more soluble compounds were produced with calcium (compounds with NO_3^-, Cl^-, I^-).

Other forms of nitrogen loss

Nitrite ions in soil can be non-biologically converted into various nitrogenous gases, such as N_2, NO, NO_2, and N_2O, by a process called chemodenitrification (Chalk and Smith, 1983; Haynes and Sherlock, 1986). This process is only significant where NO_2^- accumulates in soils, which is not frequent. However, high pH conditions accompanied by high levels of NH_4^+, both of which inhibit *Nitrobacter winogradskyi*, can result in increasing NO_2^- levels.

Some forms of N can be adsorbed onto soil particles (Cameron and Haynes, 1986; Savant and De Datta, 1982). This most commonly occurs with NH_4^+, which bonds to the negatively charged sites on clay minerals or organic matter. This process is generally reversible. In addition, NH_4^+ can be fixed within the structure of 2:1 clay minerals, such as vermiculite and montmorillonite, in a nonexchangeable way, which is generally not reversible. Nitrate ions, which have negative charges, are generally not fixed nor are they adsorbed in the soil, because of the low concentration of positive sites on clay minerals or organic matter.

Nitrate leaching can be major problem on agricultural soils (Cameron and Haynes, 1986; Keeney and Sahrawat, 1986). Being soluble in soil water, the NO_3^- ion moves through the profile with soil water. Except for that taken up by plants, most NO_3^--N is generally believed to be lost in this way. The net effect of nitrification followed by substantial leaching is a loss of N from the rhizosphere. Increased rainfall or irrigation can lead to greater leaching losses, although evapotranspirative needs during the growing season temper the loss somewhat. The intensity of precipitation

can negate the effect of evapotranspiration. Poorly structured or light-textured soils are more prone to leaching than well-structured or heavy soils. Since many soils have little anion exchange capacity, little NO_3^- is adsorbed onto soil particles. Heavy application of fertilizer-N can increase leaching losses.

Finally, much N is lost through soil erosion and surface runoff (Cameron and Haynes, 1986). Most of this loss is water-borne, although about 20% is because of wind erosion. Frequently, however, erosion and runoff do not represent an actual N loss, but rather a movement of N from one place to another. Unfortunately, this movement is often away from the place desired, that is, away from heavily fertilized agricultural land, and frequently into water sources.

Nitrogen Losses in Rice Production

Plant recovery of fertilizer N is seldom more than 30-40% of what was applied under normal conditions, and not more than 60-65% under optimum conditions (De Datta, 1981). Patrick and Reddy (1976) reported N recoveries from ammonium sulfate of 49% when N was applied early by deep placement, 51% when N was applied as an early topdress, and 61% when N application was split as early and midseason topdresses. Nitrogen losses from the plant-soil system ranged from 14 to 25%.

Three mechanisms are frequently cited as the means by which N is lost in rice production. These are (1) denitrification of NO_3^-, (2) ammonia volatilization, and (3) NH_3, N_2, or N_2O emission from plant tissue. The conditions vary under which these occur and frequently they occur simultaneously in the same field.

Denitrification losses

Reddy and Patrick (1986) reviewed the literature on denitrification losses in flooded soils. They reported that denitrification in flooded soils occurs primarily in the reduced soil layer that is deficient in O_2. However, this occurs only where temporary reduced conditions exist or where the soils are less intensively reduced (Eh values of 200 to 300 mV). Temporary reduced conditions exist when there is frequent flooding and drying of the soil, as is the case in the pinpoint flood system and is sometimes the case in rainfed lowland rice. Less intensively reduced conditions exist in the proximity of the oxidized-reduced interface, near the soil surface and around rice roots. In more reduced conditions, dissimilatory reduction is favored, that is, NO_3^- is converted to NH_4^+.

In order for denitrification to occur, a supply of NO_3^- is needed. Nitrate-containing fertilizers and nitrification of NH_4^+ provide the NO_3^- needed. Ammonium diffuses from the reduced soil layer to the oxidized soil layer. Nitrification occurs in the floodwater, in the oxidized soil layer, and in the rhizosphere. When the oxidized soil layer is thick, such as in soils with low organic matter content, most NH_4^+ is converted to NO_3^-. When the oxidized soil layer is thin, such as in soils with high organic matter content, some NH_4^+ will not be nitrified. The NO_3^- formed diffuses to the reduced soil layers, where it can then be denitrified. Nitrate diffusion and denitrification occur rapidly, whereas NH_4^+ diffusion and nitrification limit the rate and amount of N loss by denitrification.

Since 1984, the major advance in denitrification studies has been the ability to directly measure N_2 and N_2O fluxes under field conditions. Direct measurement of N_2 produced by denitrification was particularly difficult because the small quantities of N_2 produced are combined with air that is already 78% N_2 (Buresh and De Datta, 1990), and the N_2 produced from ^{15}N is not randomly distributed with atmospheric N_2 (Craswell *et al.*, 1985). Buresh and Austin (1988) reported a method which used highly ^{15}N-enriched urea and plexiglass chambers in the field. The gasses collected were analyzed using methods developed by Craswell *et al.* (1985) and Strong *et al.* (1987). Buresh and Austin (1988) found that 1% or less of the incorporated urea was evolved as N_2 or N_2O, although they indicated that their method underestimated N losses by denitrification. Lindau *et al.* (1988) suggested that the underestimation was caused by soil entrapment of applied N during denitrification. In a subsequent study using the same methodologies, Buresh *et al.* (1991) reported similarly low denitrification losses, and found that ammonia volatilization accounted for greater amounts of N losses.

Ammonia volatilization losses

Fillery and Vlek (1986) reviewed the literature on losses from ammonia volatilization in flooded rice fields. They reported that the quantity of NH_3 in floodwater represents the potential for ammonia volatilization, while actual NH_3 loss is dependent on wind speed and on the equilibrium vapor pressure of NH_3 in floodwater. The quantity of NH_3 in floodwater increases approximately tenfold per unit increase in pH between 7.5 and 9.0, and increases linearly with temperature. Fillery and Vlek

(1986) reported ammonia volatilization losses of up to 50% when urea-N was applied, but only 11% when ammonium sulfate was applied. Since ammonia volatilization is an acidifying process, continued volatilization causes a reduction in pH, thus reducing the potential for further volatilization. While urea and ammonium sulfate are sources of floodwater NH_3, urea hydrolysis buffers H^+ production in the ammonium-to-ammonia reaction, thus maintaining alkalinity. Therefore, when urea was applied, more NH_3 was volatilized than when ammonium sulfate was applied.

Fillery and Vlek (1986) also reported on the effects of management practices on ammonia volatilization. Lower NH_3 loss was detected when broadcast urea was incorporated into puddled but drained soil than when applied directly into the floodwater. This was primarily because of lower N concentration in floodwater after flooding the field in which urea had been incorporated. When urea was applied directly to floodwater at PI, lower rates of NH_3 loss were recorded than when urea was applied into floodwater prior to PI. It is possible that the rice crop shaded the floodwater. Shading suppressed photosynthetically driven pH increases, and restricted air exchange at the floodwater surface. Wells and Turner (1984) suggested that the reduction in N loss when urea was applied at PI was because a sufficient root mass had formed at the soil surface allowing for rapid N uptake by the plant.

Other than the primary factors which affect ammonia volatilization, Jayaweera and Mikkelsen (1991) identified some secondary factors. Urea and ammonium sulfate produce different floodwater NH_4^+ levels at times, approximately the same at other times. In either case, however, NH_3 fluxes were detected immediately after ammonium

sulfate application and reached a maximum at that time. Ammonia fluxes were detected 2 to 4 hours after the application of urea and reached a maximum only a few days later. Reduced N application rates resulted in reduced ammonia volatilization. Incorporation or deep placement of fertilizer also resulted in reduced ammonia volatilization. Soils with higher CEC had reduced ammonia volatilization. All of these secondary factors affected floodwater NH_4^+ concentration, thus affecting the concentration of the substrate for volatilization.

Gaseous N losses from plant foliage

Ammonia emission from the foliage of growing plants is known to occur, but the physiological and biochemical processes affecting that emission are not clear (Farquhar *et al.*, 1983; Schjøerring, 1991). Schjøerring (1991) reviewed the literature on NH_3 emission from plants, and found that the rate and direction of plant NH_3 fluxes are functions of the NH_3 partial pressure gradient between the stomatal cavities and the ambient atmosphere. When the intercellular partial pressure of NH_3 is greater than the ambient partial pressure, NH_3 emission will occur. Intercellular NH_3 results from the imbalance between NH_4^+-releasing processes, such as protein degradation and photorespiration reactions, and NH_4^+-assimilating processes, such as the formation of glutamate and glutamine, and reactions involving glutamate synthase and glutamine synthetase. High light, ample moisture, and high levels of N were each reported to increase stomatal conductance to NH_3, and increases in temperature resulted in a corresponding increase in photorespiration and NH_3 flux.

Emissions from crop plants appeared to take place mainly during the reproductive growth phase (between anthesis and maturity), and increased with the N status of plant tissue (Schjøerring, 1991; Wetselaar and Farquhar, 1980). During the reproductive phase, proteins break down in senescing leaves, producing NH_3. The increase in available NH_3 makes gaseous loss from plant tissue possible.

da Silva and Stutte (1981) found that NH_3 emissions from rice plants varied among growth stages. They found high N losses at tillering and heading, but low N losses at PI. The Y-leaf lost more N than other leaves, possibly because it was more active metabolically. The Y-leaf is the most recently matured leaf of a rice plant tiller. Transpiration rate and N loss were correlated, and transpiration rate decreased with age, possibly because stomatal resistance increased with age. In a second study, Stutte and da Silva (1981) found that the rate of N loss increased as the temperature increased from 30-35°C. In addition, they found differences in N loss by variety, although all varieties showed a decrease in N loss between tillering and PI, and an increase in N loss between PI and heading. Variety differences in N loss were suggested as a reason for differential response to N fertilization strategies (da Silva and Stutte, 1981). Mosier *et al.* (1990) reported that rice plants acted as a conduit for efflux of N_2 and N_2O that was produced by denitrification but trapped in the flooded soil. They found little N-loss directly from denitrification, but found that ammonia volatilization was the main N-loss mechanism.

Added Nitrogen Interactions

Added nitrogen interactions, or the priming effect, have been described in a review by Jenkinson *et al.* (1985). Added nitrogen interactions are 'real' when fertilizer N causes an acceleration in mineralization of soil organic N, or an increase in root growth such that the soil profile explored by roots is increased. Both increase the N available to plants or to soil organisms. Neither appear to be common. More common are 'apparent' added nitrogen interactions, which occur when application of fertilizer N results in displacement reactions or pool substitution. Displacement reactions are situations where fertilizer N displaces exchangeable native soil N that is not directly accessible to plants or to soil organisms, making the native N available. Pool substitution occurs when fertilizer N replaces native soil N that is available to plants or to soil organisms. Neither increase the N that plants or soil organisms can harvest, however, they do alter the source of that N. For example, if isotopically labeled N is used as a fertilizer source, the N accumulated by the plant will not contain the same percentage of isotopically labeled N as was in the soil solution, but rather will contain more native soil N because of displacement and substitution by native N for labeled N. Hart *et al.* (1986) demonstrated such a positive added nitrogen interaction, where labeled fertilizer N increased the uptake of unlabeled soil N by replacing soil N that would have otherwise been immobilized. Care must be taken in the interpretation of such experiments, because fertilizer use efficiency will be lower than if the same experiment had been conducted with non-isotopic procedures.

De Datta and Broadbent (1988) and Jenkinson *et al.* (1985) stated that the effect of fertilizer N on root growth is minimal. However, Azam *et al.* (1991) reported an increase in root dry matter when fertilizer N was applied to five varieties of flooded rice, and showed an associated increase in soil N accumulation. Their results suggest that a real added nitrogen interaction took place. In a second study (Azam *et al.*, 1992), results under non-saline conditions were similar and they were able to demonstrate that the added nitrogen interaction increased with increasing rates of fertilizer N.

Nitrogen Management in Rice

Due to the N transformations and losses described above, only a small portion of N applied as fertilizer will actually contribute to grain production. In order to control the transformations and mitigate the losses, strategies for optimum N placement and timing, effective cultural methods, and efficient fertilizer sources have been developed.

Nitrogen Placement

There is almost universal agreement that deep placement or shallow incorporation of fertilizer N results in lower N losses, greater N accumulation, and higher grain yield than surface application on wet or flooded soils (Cao *et al.*, 1983, 1984; De Datta, 1986; De Datta *et al.*, 1988; De Datta *et al.*, 1989; Heenan and Bacon, 1989; Rao and Shinde, 1985; Schnier *et al.*, 1990; Vlek and Byrnes, 1986; Wells and Turner, 1984). This is because the amount of ammoniacal-N in the standing floodwater is reduced by incorporation, thus controlling the potential for ammonia volatilization

losses, and the potential for nitrification is lessened when N is placed in the reduced zone of the soil profile (Wells and Turner, 1984). In field studies in the Philippines, De Datta *et al.* (1988) reported that deep placement of urea resulted in lower N loss and higher grain yield than when urea was incorporated into wet soil, which in turn had lower N loss and higher grain yield than when urea was broadcast into the floodwater. Heenan and Bacon (1989) reported that urea broadcast onto wet soil resulted in lower grain yield and lower N accumulation than urea broadcast onto or incorporated into dry soil. Highest grain yield and N accumulation were found when N fertilizer was drilled into the soil, but the drill depth was not significant. Schnier *et al.* (1990) demonstrated that N efficiency can be improved with different methods of incorporation or deep placement. They found that band placement of liquid urea and urea supergranule point placement was superior to the conventional broadcast and incorporation method. Previously, Cao *et al.* (1983) showed that uniform placement and point placement of urea supergranules produced higher grain yield than band placement.

Nitrogen Timing

In order to improve fertilizer N efficiency, split fertilizer applications which coincide with plant demand have been suggested. Results of studies on the effectiveness of split applications do not agree. Reddy and Patrick (1976) reported no difference in grain and straw yield between preflood application of N and a preflood-midseason split application when the topdress was applied before PI. However, they saw a reduction in grain yield when the topdress was applied at PI. They did show that the split application resulted in greater total N in the grain. In a second study (Reddy

and Patrick, 1978), grain yield did not vary between preflood N and a preflood-midseason split application when ammonium sulfate was the N source, but the preflood-midseason split application had greater grain yield when urea was the N source. As before, they noted greater N accumulation in grain and straw when a split application was applied. Westcott *et al.* (1986) reported that there were no differences in straw or grain yield between preplant or preflood N and split applications at PI. However, recovery of fertilizer N was greater with a split application than with only preplant or preflood N. Norman *et al.* (1990) found that a three-way split application generally did not affect grain yield. Results from Wells *et al.* (1989) were as varied as the locations of the experiments in Arkansas, California, Louisiana, Mississippi, and Texas. However, a three-way split application frequently resulted in greater N efficiency and grain yield than a preplant or preflood N application. Norman *et al.* (1989) found higher grain yield and N accumulation with a three-way split N application at panicle differentiation (PD) and PD plus 10 days than with only an early season application. Bollich *et al.* (1991b, 1993a) reported no difference in grain yield between a single application or a two-way split in 1991 and 1993, but a significant yield reduction in a three-way split when the third application was at PD. However, in 1992, they found that both a two-way and a three-way split application resulted in lower grain yield than a single application (Bollich *et al.*, 1994a).

No clear conclusions can be draw from these studies. The implication is that a preplant- or preflood-midseason split application of N generally results in greater N accumulation but not necessarily greater grain yield than when all N is applied early.

Under certain conditions, however, the increase in N accumulation does result in an increase in grain yield. There was generally no decrease in grain yield due to a split N application, except when part of the midseason application was at PD or later.

Little information is available on plant growth and N accumulation after a midseason application. In an experiment comparing seeding methods and split application of N, De Datta *et al.* (1988) reported differences in the pattern of N accumulation by the plant at high N rates, but no difference at low N rates. Wilson *et al.* (1989) examined N accumulation by the plant from a three-way split application. Their results showed that most N from a midseason application was recovered within three days after application. Except for their early seasons results, recovery appeared to increase and then decrease over time, rather than following a linear or curvilinear pattern.

Because of the confusion about the value of split N applications, there is need to study the effect of midseason N on plant dry matter yield, root growth, and N accumulation. In addition, the length of time during which midseason N is accumulated needs to be determined. The present study addressed these needs.

Nitrogen Sources

Urea ($CO(NH_2)_2$), ammonium sulfate, or other non-NO_3^--N fertilizers are generally used with flooded rice, since NO_3^--N cannot be retained in a submerged soil without denitrification losses (Brandon and Wells, 1986). Wells and Turner (1984) suggested that NO_3^--N could be almost as effective as NH_4^+-N when applied in small amounts (less than 30 kg ha^{-1}) at PI after a root mat had formed at the soil surface such

that plants can rapidly absorb the applied N. However, Wilson *et al.* (1994) compared granular urea to a urea-ammonium nitrate solution applied in a three-way split, and found that the highest fertilizer N accumulation and grain yield occurred when granular urea was the N source. An early study by Patrick *et al.* (1967) reported little difference between ammonium sulfate and urea. A later study (Reddy and Patrick, 1978) done with ^{15}N in microplots, again found no differences in grain or straw yields for the two N sources. Neither study took into account the possible contribution of the sulfur in the ammonium sulfate. Blair (1987) reported a response to sulfur applied with N in flooded soils, and indicated that S deficiency has the same effect as N deficiency. Both reduce grain yield by reducing tillering, number of panicles, grains per panicle, and grain weight. Sarkar *et al.* (1978) found the highest N accumulation and fertilizer use efficiency from ammonium sulfate in a greenhouse study comparing urea, anhydrous ammonia, and ammonium sulfate. Grain yields were highest at 60 kg N ha^{-1} when ammonium sulfate was the N source, and highest at 120 kg N ha^{-1} when urea was the N source. Phongpan *et al.* (1988), working on an acid sulfate soil, found no differences in grain and straw yields between urea and ammonium sulfate at a low N rate (800 mg N per 5 kg soil), but at higher N rates (1600 and 2400 mg N per 5 kg soil), urea consistently produced higher yields than ammonium sulfate. Fertilizer N recovery, however, was greater when ammonium sulfate was used rather than urea at transplanting. At PI, greater N recovery was obtained with urea. Previous studies were not in agreement about the effectiveness of urea and ammonium sulfate, they used very small microplots that might not provide field-reliable data, and the effect of S in

ammonium sulfate was not considered. Therefore, in the present study, it was necessary to compare ammonium sulfate and urea once again, taking into account the possible contribution of S, and looking at the effect of retainers and microplot size on field experiments using ^{15}N.

Urease and Nitrification Inhibitors

Hydrolysis of broadcast urea increases NH_3 concentration in floodwater, resulting in an increase in ammonia volatilization. This is a particular problem in parts of Asia, where many rice farmers broadcast urea without subsequent incorporation into the soil (Buresh *et al.*, 1988c). It is not a problem where urea is broadcast preflood or is incorporated into the soil. Urease allows urea hydrolysis to take place, and urease inhibitors, particularly phenyl phosphorodiamidate (PPD) and N-(n-butyl) thiophosphoric triamide (NBPT), have been used to control that reaction. Buresh *et al.* (1988b) reported that PPD delayed NH_3 buildup in floodwater, and NBPT prevented such a buildup. Luo Qui-Xiang *et al.* (1994) reported that both PPD and NBPT delayed NH_3 buildup, and that mixtures of PPD and NBPT were more effective than either inhibitor alone. Rao and Ghai (1986) found that PPD delayed urea hydrolysis by 3-4 days, and reduced ammonia volatilization from 45 to 8.5%. Other authors also reported a delay in urea hydrolysis because of PPD (Fillery *et al.*, 1986; Pedrazzini *et al.*, 1987; Simpson *et al.*, 1985). Byrnes *et al.* (1983) reported that PPD reduced ammonia volatilization losses from 31 to <5% of the N added, and showed that 1% PPD to urea (kg kg^{-1}) was as effective as 5% PPD to urea in delaying urea hydrolysis, lowering floodwater pH, reducing floodwater NH_3 concentration, and decreasing

ammonia volatilization. Lu *et al.* (1989) found that in oxidized soil, 57% of added urea was still present 5 days after NBPT addition, while only 31% was present after PPD addition. In reduced soil, 4% of added urea was still present 5 days after NBPT addition, while 56% was present after PPD addition. This indicates that both NBPT and PPD are effective urease inhibitors under oxidized soil conditions, such as at the surface of flooded soils to which urea is broadcast, but that NBPT is a better urease inhibitor than PPD. The reduction in ammonia volatilization associated with use of NBPT or PPD translated into increased grain yields on a silty clay soil in the Philippines but not on a clay soil (Buresh *et al.*, 1988c). A greater increase in grain yield with increased N rate was seen when PPD was used than when not used (Buresh *et al.*, 1988a). In a 77-day greenhouse study, Byrnes *et al.* (1983) reported that PPD increased N recovery by the plant 10-15% while reducing dry matter. They attributed the decrease in dry matter to delayed growth associated with an increase in available N and poor lighting. In a Louisiana study with pre-flood urea-N (Bollich *et al.*, 1990b, 1990c), NBPT was shown to have no effect on urea performance or grain yield. That is consistent with expectations, since the NH_3 hydrolyzed from urea would not be exposed to volatilization within the soil profile.

When urea or other ammoniacal fertilizers are broadcast preflood or incorporated in a soil with low organic matter content, as is common in rice growing areas of the United States of America, much of the NH_4^+ is nitrified to NO_3^- in the oxidized layer of the flooded soil profile, and the NO_3^- can then diffuse to the reduced layers of the profile where denitrification can occur. In order to prevent denitrification

losses, various nitrification inhibitors have been used, such as dicyandiamid (DCD), coated calcium carbide (CCC), 2 chloro-6 (tricholoromethyl) pyridine (Nitropyrin or N-serve[1]), and 5-ethoxy-e-(trichloromethyl)-1,2,4-thiodiazole (Dwell[2]). Hauck (1984) reported that N-serve functioned best with anhydrous ammonia, but was not suitable with urea because of its high vapor pressure. However, other studies (Brandon *et al.*, 1981; Das and Chatterjee, 1980; Thomas and Prasad, 1987) reported that N-serve with urea produced a higher grain yield than urea alone. Dwell with urea did not produce a higher grain yield than urea alone (Brandon *et al.*, 1981). Little research has addressed the effects of CCC, however two Louisiana studies (Bollich *et al.*, 1990c, 1991c) indicate its potential at inhibiting nitrification and increasing grain yield. Norman and Wells (1989) showed that DCD is able to slow nitrification in drained soils, resulting in 2.5 times more fertilizer-derived NH_4^+ than when DCD was not used. Norman *et al.* (1989) reported higher grain and total dry matter yield, and higher total N and ^{15}N-labeled accumulation by rice plants when DCD was used with urea. However, Wells *et al.* (1989) warned that DCD was advantageous only if flooding was delayed at least 14 days after urea application, and Simpson *et al.* (1985) found no effect on NO_3^- concentration in floodwater due to DCD..

Cultural Methods

Methods of rice cultivation differ in the various rice growing areas of the world because of pests, fertility management, the availability of irrigation water, the

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[1]N-serve is a trademark of Dow Chemical U.S.A.

[2]Dwell is a trademark of Olin Corp.

availability of farm implements, and local traditions. About 10% of the world's rice is grown in freely drained fields where there is adequate rain (upland rice), a practice common in Africa and Latin America (Mikkelsen and De Datta, 1991; Vlek and Byrnes, 1986). Irrigated flooded rice is common in China and subtropical areas, while rainfed flooded rice is common in South and Southeast Asia (Vlek and Byrnes, 1986).

In Asia, irrigated and rainfed flooded rice is either transplanted or direct-seeded (De Datta, 1986; Fillery and Vlek, 1986; Mikkelsen and De Datta, 1991; Schnier *et al.*, 1990; Vlek and Byrnes, 1986). In either case, saturated soil is worked either by a hand tractor or a draft animal until the soil is puddled. Puddled soil has uniform soil aggregates, few macropores, a reduction in trapped gasses, and an increase in water-holding capacity (Mikkelsen and De Datta, 1991). Bunds are built and maintained to hold water in the paddies. It is recommended that urea then be incorporated into the puddled soil immediately before transplanting rice or broadcasting seed (De Datta, 1986). Frequently, farmers will broadcast urea into floodwater two to four weeks after transplanting or seeding. Transplanted rice is spaced widely and depends on varieties with high tillering capacity, and is often planted during the monsoon. Direct-seeded rice is pregerminated and broadcast onto fields without much standing water (Mikkelsen and De Datta, 1991). In the southern United States, rice is either DS or WS (Bagent *et al.*, 1987; Brandon and Wells, 1986; Helms, 1984; Reddy and Patrick, 1986; Westcott *et al.*, 1986). In DS , the dry seedbed is tilled so that it is smooth and free of clods. Rice is DS or broadcast-seeded and incorporated. The field is flushed to make a moist seedbed, but not flooded. At approximately the four- or five-leaf stage of

growth, N is applied to the soil surface, and the field is flooded beginning the next day and the floodwater is maintained until two to three weeks prior to harvest. Urea, ammonium sulfate, or other non-NO_3^--N fertilizer is used, since NO_3^--N is subject to denitrification losses under submerged conditions. Fertilizer N is carried through the soil profile by the percolating floodwater, thus reducing the amount of ammoniacal-N in the standing floodwater, and controlling the potential for ammonia volatilization losses. Helms (1984) lists other advantages and also disadvantages of DS. Advantages include (1) a more uniform rice stand with less seed required than for WS rice, (2) slightly less cost per acre when DS due to less water and airplane use, and (3) less lodging at harvest. Disadvantages include (1) germination of terrestrial weeds that can tolerate later flooding, especially red rice (*Oryza sativa*), (2) slower dry-matter accumulation in early stages of growth because of greater microsite temperature fluctuations, (3) unavailability of nutrients, particularly N, in early stages of growth, and (4) slightly delayed crop maturity.

In water-seeded, the dry seedbed is tilled to provide a slightly cloddy or grooved soil surface without large clods, raised areas, or depressions. Nitrogen fertilizer is broadcast and incorporated into the prepared seedbed. The N sources are the same as in DS, for the same reasons. The field is then flooded. Presoaked seed (soaked for about 48 hours and drained for about 48 hours) is aerially broadcast into the flooded field. Presoaked seed more readily sinks to the bottom of the floodwater and does not drift across the cloddy or grooved soil surface. The floodwater is maintained until two to three weeks prior to harvest, unless a pinpoint WS is used. In

the pinpoint system, the floodwater is drained one to five days after seeding, but the soil remains saturated, allowing better seedling anchorage, then the field is reflooded a few days later and the floodwater is maintained until two to three weeks prior to harvest. Helms (1984) lists the advantages and disadvantages of WS. Advantages include (1) control of terrestrial weeds that can tolerate later flooding, especially red rice, (2) more rapid seedling growth because of a stable temperature in the seedling microclimate, and (3) increased nutrient availability, particularly N, because of early application of N and changes in the oxidation-reduction status in the soil. Disadvantages include (1) difficulty is establishing a uniform stand, (2) need for higher seeding rates because of higher seedling mortality due to lack of soil coverage of seed, seedling diseases, and insects, and (3) additional logistics required for presoaking seed and aerial broadcasting. In addition, WS is better adapted than DS to the naturally wet conditions of South Louisiana. Many of the later operations of DS cannot be performed if the soil remains wet after the initial flush. In both DS and WS, any midseason N is applied into the floodwater at PI after a root mat has formed at the soil surface, and rapid uptake by the rice plant is able to limit losses due to denitrification and ammonia volatilization (Wells and Turner, 1984). While numerous studies have considered rice production under each system, no study has compared the two systems. There is a need to determine the seasonal accumulation of rice under each system and make appropriate comparisons. This need was addressed in the current study.

Variety Selection

De Datta (1986) reported that differences in N efficiency have been detected among rice varieties in studies in the Philippines. Numerous experiment station reports have shown similar differences by variety (Bollich *et al.*, 1994b; Norman *et al.*, 1995). Many researchers have suggested that semi-dwarf varieties require higher N rates than taller varieties (Brandon and Wells, 1986; Guindo *et al.*, 1994b; Roberts, *et al.*, 1993; Wells and Turner, 1984), although others did not see the same phenomenon (Norman, *et al.*, 1992b). Their conclusions were generally based on a comparison of one variety of each type.

Since soil is the principle source of N in lowland rice production, varieties which take up or use N more efficiently should be used (De Datta, 1986). Methods for evaluating N efficiency in rice genotypes have been proposed (Broadbent *et al.*, 1987; De Datta and Broadbent, 1988). They suggested measuring several plant parameters that relate to N use and efficiency, and to rate genotypes according to these parameters. Senanayake *et al.* (1991) used electron microscopy to identify differences in growth among rice varieties, and suggested that N topdressing might need to vary among early maturing and late maturing varieties. Laza *et al.* (1993) found differences in chloroplast ultrastructure among rice varieties, suggesting that increases in chloroplast size was related to increases in chlorophyll, protein content, and dry weight.

Little research has been conducted to determine N use by variety-type or mechanisms of N use that would differentiate varieties and make one more variety

more N-efficient than another. Therefore, it is necessary to study the relationships between N accumulation and variety-type, and that was addressed in the present study.

CHAPTER 1

RICE PLANT GROWTH AND NITROGEN ACCUMULATION IN DRILL-SEEDED AND WATER-SEEDED CULTURE

Introduction

Nitrogen is a major nutrient in flooded rice culture throughout the world. However, fertilizer N recovery is commonly not more than 30-40%, and seldom more than 60-65% under optimum conditions (De Datta, 1981). To develop efficient N fertilization strategies, both plant N demand and soil N availability need to be considered. Research has addressed the availability of soil N (Kenney and Sahrawat, 1986; Reddy and Patrick, 1983; Savant and De Datta, 1982), however, plant N demand has received less attention.

Several studies in which unlabeled N (^{14}N) or obsolete cultivars were used, have addressed N accumulation by rice. Reyes *et al.* (1962) reported that rice grown in the Philippines showed a marked accumulation of N from boot stage to maturity, with 60% of the total N in the grain at maturity. They found that N rate had no effect on the dry matter or N content of various plant parts, and attributed that to the inherent fertility of the field. Ishizuka (1965) reviewed nutrient accumulation data from studies done in Asia by various researchers, who used different cultivars, methods, climates, and soils, with unidentified N sources. A sigmoidal pattern of N uptake was reported, with remobilization from straw to grain in later stages of growth. Sims and Place (1968) conducted research in Arkansas with different rates of anhydrous ammonia. They found that the amounts of N taken up generally paralleled dry matter production and

increased with the age of the plant. Moore *et al.* (1981) reported that N uptake followed patterns similar to dry matter production, which was sigmoidal. Nitrogen was remobilized from straw to grain until grain formation was complete.

Recent studies in Arkansas with labeled N (^{15}N) have provided new information on fertilizer and total N uptake. Norman *et al.* (1992a) reported an increase in total N accumulation throughout the season from a preflood application. Fertilizer N accumulation peaked at 55 days after emergence and then declined. When N was applied in split applications, fertilizer N accumulation peaked at 69 days after emergence, but did not exceed the amount of a single preflood application. Native soil N provided the needed N late in the season. Nitrogen accumulated in grain after heading, decreased in other plant parts, and greatest fertilizer N recoveries occurred with a single preflood application. Guindo *et al.* (1994a) reported similar results, and found maximum total N accumulation at 21 days after heading from a preflood application, and at maturity for a split application.

Root studies on flooded rice have been infrequent because of labor intensity and the difficulty in acquiring useful field measurements. Morita and Abe (1994) reviewed rice root research and reported that although the development of the root system of a rice plant paralleled that of the shoot, the shape of the root system was almost constant throughout the developmental stages. They found a linear relationship between the log of shoot weight and the log of root weight, as well as a linear relationship between the log of leaf area and the log of root length. Yoshida and Hasegawa (1982) reported that plant height and rooting depth were not related. Rather, tillering habit was associated

with rooting habit. Beyrouty *et al.* (1988) and Slaton *et al.* (1990) found that root growth varied dramatically among soils and depended on the influence of soil profile characteristics, soil moisture, air temperature, changes in plant development, and genotype. Beyrouty *et al.* (1996) reported that development of root length of rice is rapid and linear during vegetative growth, maximum root length is achieved by panicle initiation or booting, and the maximum rate of root growth occurs between maximum tillering and panicle initiation. A net decrease in root length occurs between heading and grain fill.

Much of the field research in the southern United States has been conducted in DS, in which seed are planted in a prepared field, N fertilizer is applied to the soil surface at about the four-leaf stage of plant growth, and the field is then flooded. However, WS is commonly used in south Louisiana and Texas and is increasing in popularity in other areas of the southern U.S. rice belt for red rice suppression. In WS, N fertilizer is incorporated into the soil prior to flooding, after which pregerminated seed are broadcast into the floodwater. Published results on seasonal N accumulation in WS are unavailable. Therefore, the objectives of this study were to characterize plant growth and N accumulation by rice at different stages of development, and to compare these factors in DS and WS.

Materials and Methods

Field experiments were conducted in 1993-1995 at the Rice Research Station in Crowley, LA, in a Crowley silt loam soil (Table 1.1). Both WS and DS methods were

Table 1.1. Initial soil chemical and physical properties of Crowley silt loam at the Rice Research Station, Crowley, LA.

Soil property	1993	1994	1995
pH	6.1	5.8	6.5
Organic matter (%)	1.24	1.26	1.13
Extractable P (mg kg^{-1})[1]	47	24	78
Exchangeable Na (mg kg^{-1})[2]	77	70	98
Exchangeable K (mg kg^{-1})[2]	58	57	66
Exchangeable Ca (mg kg^{-1})[2]	1221	1065	1235
Exchangeable Mg (mg kg^{-1})[2]	202	173	201
Extractable Zn (mg kg^{-1})[3]	0.89	2.24	7.45
Extractable S (mg kg^{-1})[4]	7.09	16.37	9.09
Extractable As (mg kg^{-1})[5]	1.95	11.51	0.71
Extractable Fe (mg kg^{-1})[3]	61.55	122	182.8

[1]Bray-2
[2]Ammonium acetate at pH 7.0
[3]Diethylene triamine pentaacetic acid
[4]Acetic acid and ammonium acetate
[5]Hydrochloric acid

used each year. In WS, 75 x 75 cm microplots with stainless steel retainers were established, with approximately 10 cm of retainer wall below and 20 cm above the soil surface. Fertilizer N (2.60 atom% ^{15}N in 1993, 2.22 atom% ^{15}N in 1994, and 2.09 atom% ^{15}N in 1995) was applied as urea to all microplots approximately 3 cm below the soil surface at 151 kg N ha^{-1} and the microplots were then immediately flooded. Two days later, pregerminated 'Cypress' rice was broadcast into the floodwater of the microplots at 168 kg ha^{-1}. In DS, 'Cypress' rice was seeded in 17.8 cm rows at 112 kg ha^{-1}. Microplots (75 x 75 cm) with stainless steel retainers were established at the four-leaf stage of plant development, with approximately 10 cm of retainer wall below the soil surface and 20 cm above. Fertilizer N (2.05 atom% ^{15}N in 1993, 2.22 atom% ^{15}N in 1994, and 2.09 atom% ^{15}N in 1995) was surface applied as urea to all microplots at 151 kg N ha^{-1}. Unlabeled N was surface applied to the areas around the microplots (including areas around WS microplots) at 151 kg N ha^{-1}. The DS microplots and the surrounding field were then flooded, and the flood maintained throughout the experiment.

In 1993, the experimental design was a split plot with main plots completely randomized and subplots arranged in blocks. Seeding method was the main plot factor and harvest stage was the subplot factor. Main plots were replicated three times. Microplots were harvested at 7 and 21 days after the four-leaf stage, panicle differentiation, 90% heading and physiological maturity.

In 1994 and 1995, the experimental design was a split plot with both main plots and subplots arranged in blocks. Seeding method was the main plot factor and harvest

stage was the subplot factor. Main plots were replicated three times. Microplots were harvested at the four-leaf stage, 7, 14, and 21 days after the four-leaf stage, panicle differentiation, 90% heading, 21 days after 90% heading, and at physiological maturity.

For each microplot, all above-ground plant material was harvested by hand. In 1993, the sample at maturity was separated into straw and grain. In 1994 and 1995, the samples at 90% heading, 90% heading plus 21 days, and maturity were separated into straw and grain. Soil samples were collected with a 1.8 cm diameter hand probe to a depth of 15 cm. Two cores were collected from each plot in 1993, and four cores in 1994 and 1995. Root samples were also collected with the hand probe to a depth of 15 cm. Four cores were collected from each plot and separated into 0- to 7.5-cm and 7.5- to 15-cm depth increments.

Plant material was oven-dried at 60°C to a constant weight, while soil samples were air-dried. Samples were ground in a Wiley mill to pass a 1 mm mesh sieve. Subsamples of dried plant tissue and soils were digested in concentrated sulfuric acid. The semi-micro Kjeldahl was followed for total N determination procedure (Bremner and Mulvaney, 1982). Ethanol (95%) was distilled between samples to prevent ^{15}N contamination during distillation. Distillates were acidified with 2 mL of .08*N* acid and evaporated to dryness for ^{15}N analysis (Hauck, 1982). Nitrogen 15 atom% was determined on a Du Pont 21-621 mass spectrometer. Roots were separated from soil with a root washing system. Root length was determined with an edge discrimination technique developed by Pan and Bolton (1991), and root length densities were calculated for each sample.

Data were analyzed with the SAS System (SAS Institute, 1994). The MIXED procedure was used to determine if differences existed among seeding methods and among harvest dates, and if important interactions existed. Because numerous interactions occurred between several factors and years, data of each year were analyzed separately. The GLM procedure was used to determine if differences existed among air temperatures. Comparisons between stages of development in DS or WS, and between years for each two-week period were made with a t-test of the corresponding marginal means (with a Bonferroni correction). Assumptions for the validity of the analyses were checked.

Results and Discussion

Plant Growth

Total dry matter production was highest in 1994 and lowest in 1995 (Fig. 1.1). Total dry matter increased until 21 days after heading in both years, with the greatest increases occurring after panicle differentiation. This is consistent with the results of Guindo *et al.* (1994a), who reported that total dry matter increased until heading plus 21 days, then did not change. In 1993, total dry matter production during the early and late parts of the growing season was similar in DS and WS, with differences between methods observed during the middle of the season (Fig. 1.1). In 1994 and 1995, total dry matter was greater under WS management at heading plus 21 days, but did not differ at other stages.

After panicle differentiation, straw production continued, and grain fill began (Fig. 1.1). Except for a decrease in straw dry matter in WS in 1995, straw and grain

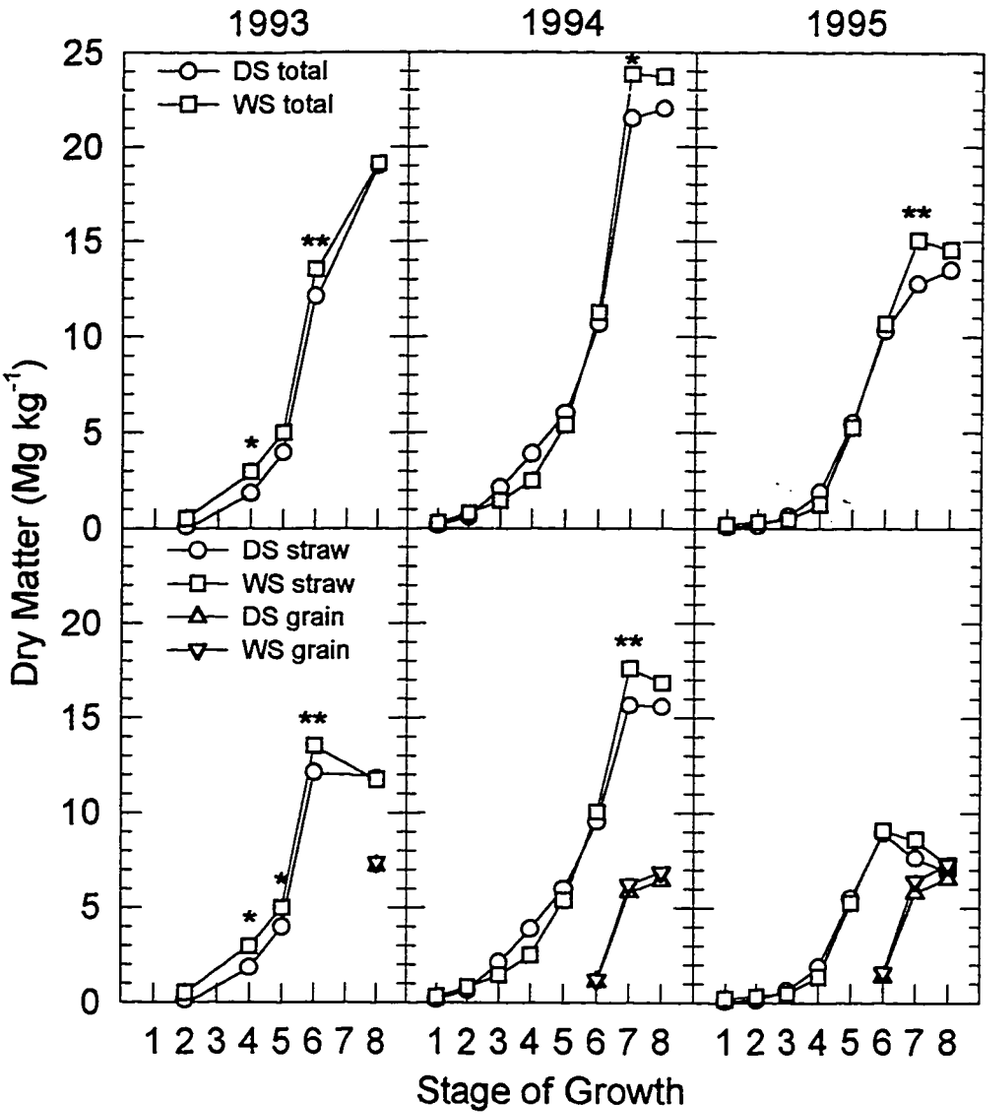

Fig. 1.1. Dry matter accumulation of rice plants grown in drill-seeded (DS) and water-seeded (WS) culture. Stages of growth are: 1=four leaf (4L), 2=4L + 7 days, 3=4L +14 days, 4= 4L + 21 days, 5=panicle differentiation, 6=90% heading, 7=90% heading + 21 days, 8=maturity. Asterisks indicate significant differences between DS and WS at α=.05 (*) or α=.01 (**).

dry matter did not change from 21 days after 90% heading to maturity in 1994 and 1995. Since grain dry matter at maturity did not differ between cultural methods within each year and from year to year at any stage of development, differences in straw dry matter accounted for differences in total dry matter between cultural methods. In 1993, straw dry matter was greater in WS than DS at the four-leaf plus 21 day, panicle differentiation, and 90% heading stages (Fig. 1.1). In 1994, straw dry matter was greater in WS than DS at the heading plus 21 day stage. Straw dry matter was the same under both DS and WS in 1995.

Patterns of root growth differed from year to year, but did not differ by cultural method in any year (Fig. 1.2). In 1993, surface root length density (0-7.5 cm depth) increased throughout the season in a linear pattern. Subsurface root length density (7.5-15 cm depth) increased until 90% heading, then remained constant or decreased. In 1994, surface and subsurface root length density increased until 90% heading and then decreased (Fig. 1.2). In 1995, surface root length density increased until panicle differentiation and then decreased. Subsurface root length density increased in WS throughout the season, while it increased in DS only until panicle differentiation and then decreased (Fig. 1.2). Morita and Abe (1994) reported that rice roots increase in both number and length until heading, then increase only in length. This partially explains the increase in surface root length density from 90% heading to maturity in 1993, although other factors must have contributed. The decrease in root length density from 90% heading to maturity in 1994 can be explained by root death exceeding new root growth. Beyrouty *et al.* (1988, 1996) found maximum root

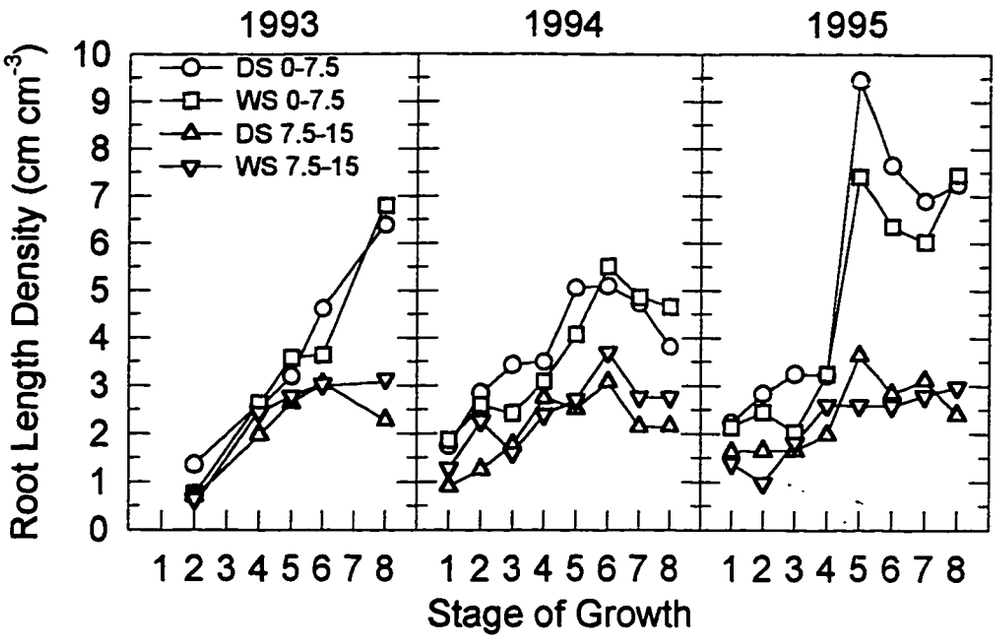

Fig. 1.2. Root growth of rice plants for two rooting depths (0-7.5 and 7.5-15 cm) grown in drill-seeded (DS) and water-seeded (WS) culture. Stages of growth are: 1=four leaf (4L), 2=4L + 7 days, 3=4L +14 days, 4= 4L + 21 days, 5=panicle differentiation, 6=90% heading, 7=90% heading + 21 days, 8=maturity.

growth at panicle initiation, with root lengths either constant or declining thereafter. Root length densities found in a Crowley silt loam in Arkansas were comparable to our values. However, they found greater root length density at 15-cm depth than at 5, 25, or 35 cm.

Nitrogen Accumulation

Plants accumulated N throughout the season, although the the rate (slope) of N accumulation did not increase after panicle differentiation, except in 1994 (Fig. 1.3). Guindo *et al.* (1994a) reported similar results. Moore *et al.* (1981) reported that N uptake followed patterns similar to dry matter production in DS, but that was not seen in the present study. More N was accumulated in 1994 than 1993 or 1995, with the difference between years beginning before panicle differentiation (Fig. 1.3). In 1994 and 1995, there was no difference in N accumulation in each cultural method between heading plus 21 days and maturity.

Total N accumulation differed by cultural method only at the four-leaf plus 21 day stage (Fig. 1.3). Water-seeded plants accumulated more N in 1993, while in 1994 and 1995, DS plants accumulated more N. This difference by year was seen in both the whole plant (Fig. 1.3) and in straw (Fig. 1.4). There were no differences in total N accumulation between DS and WS early or late in the season each year.

Accumulation of N from labeled fertilizer increased until the four-leaf plus 21 day or the panicle differentiation stages, then did not change throughout the remainder of the season (Fig. 1.3). Most of the fertilizer N in the soil had been depleted by panicle differentiation in 1993 (Fig. 1.5), therefore, little was available for plant

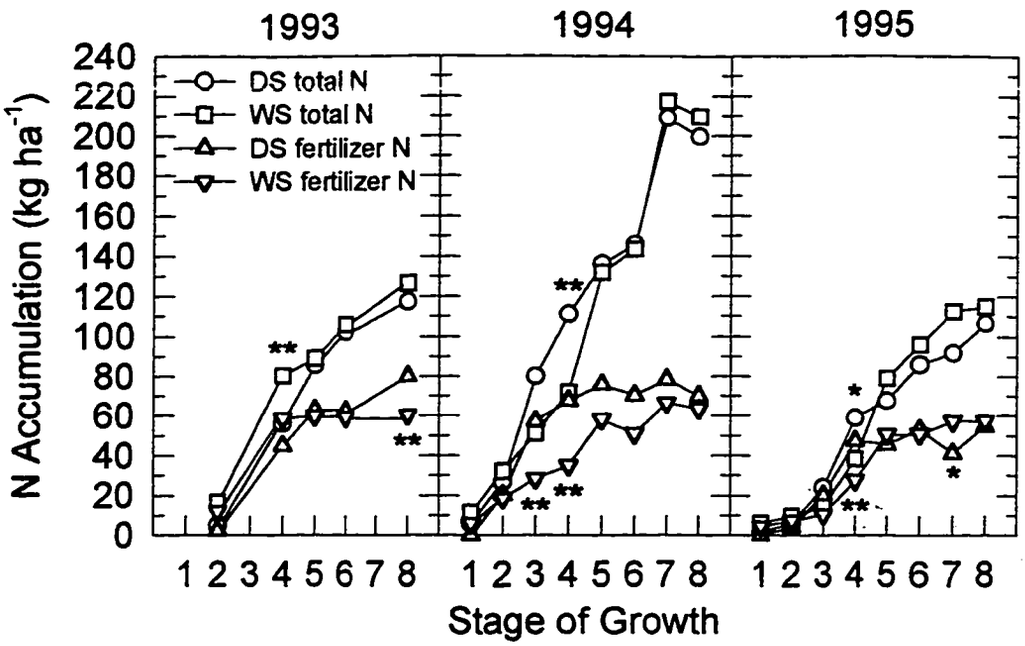

Fig. 1.3. Nitrogen (N) accumulation of rice plants grown in drill-seeded (DS) and water-seeded (WS) culture. Total N includes both fertilizer N and native soil N. Stages of growth are: 1=four leaf (4L), 2=4L + 7 days, 3=4L +14 days, 4= 4L + 21 days, 5=panicle differentiation, 6=90% heading, 7=90% heading + 21 days, 8=maturity. Asterisks above symbols indicate significant differences in total N between DS and WS at α=.05 (*) or α=.01 (**). Asterisks below symbols indicate significant differences in fertilizer N.

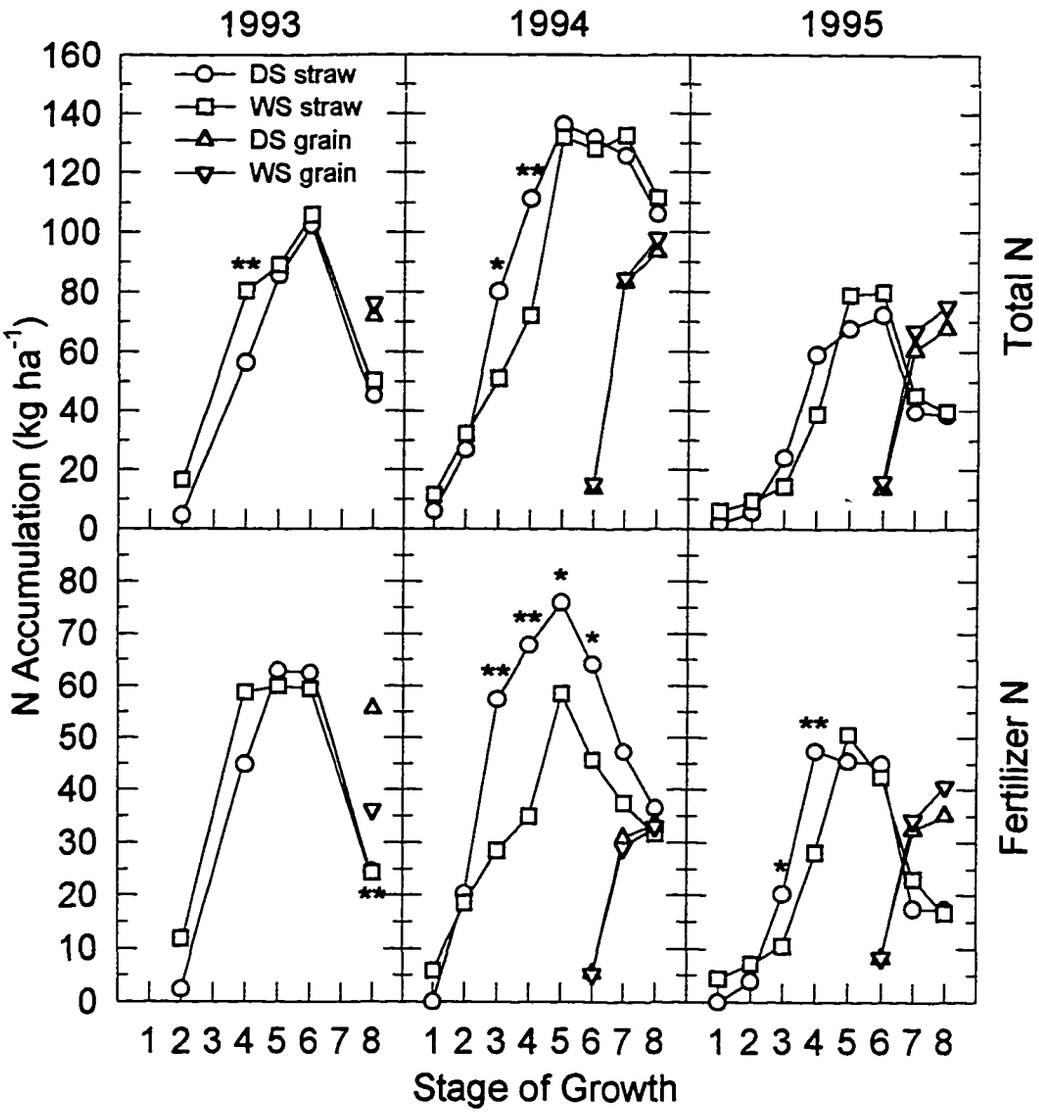

Fig. 1.4. Nitrogen (N) accumulation by straw and grain of rice plants grown in drill-seeded (DS) and water-seeded (WS) culture. Total N includes both fertilizer N and native soil N. Stages of growth are: 1=four leaf (4L), 2=4L + 7 days, 3=4L +14 days, 4= 4L + 21 days, 5=panicle differentiation, 6=90% heading, 7=90% heading + 21 days, 8=maturity. Asterisks above symbols indicate significant differences in straw total N between DS and WS at α=.05 (*) or α=.01 (**). Asterisks below symbols indicate significant differences in grain N.

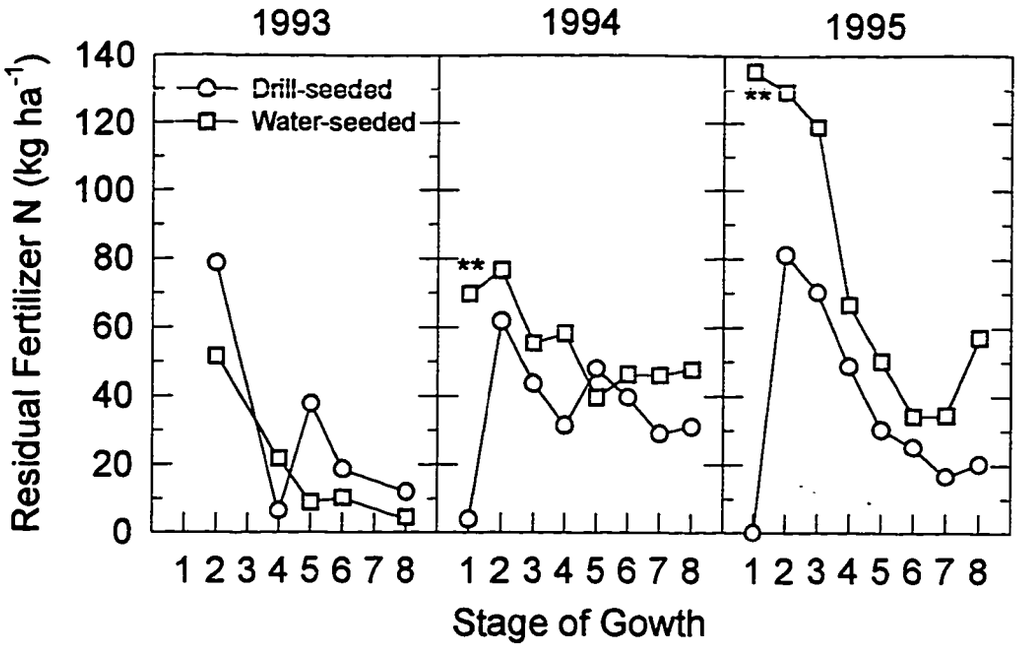

Fig. 1.5. Residual fertilizer nitrogen (N) in soil under drill-seeded (DS) and water-seeded (WS) rice culture. Stages of growth are: 1=four leaf (4L), 2=4L + 7 days, 3=4L +14 days, 4= 4L + 21 days, 5=panicle differentiation, 6=90% heading, 7=90% heading + 21 days, 8=maturity. Asterisks indicate significant differences in N between DS and WS at α=.05 (*) or α=.01 (**).

uptake. Although the pattern of fertilizer N accumulation in 1994 and 1995 was similar (Fig. 1.3), almost one-third of the applied fertilizer N was available during the latter part of those two seasons (Fig. 1.5). However, this fertilizer N was not taken up by the rice plants. A likely explanation is pool substitution, which occurs when fertilizer N exchanges with native soil N (Hart *et al*, 1986; Jenkinson *et al*, 1985). Although the amount of plant available N does not change, the source of the N does. Since this substitution occurs randomly, the effect among years and between cultural methods can differ, making it difficult to both describe and explain trends in fertilizer N accumulation. Fertilizer N in the soil was the same between cultural methods in a given year, except at the four-leaf stage in 1994 and 1995 (Fig. 1.5), when fertilizer N was applied in DS after the first dry matter harvest. Fertilizer N accumulation in plants at maturity was approximately the same from year to year (Fig. 1.3). Most N that plants accumulated by the four-leaf plus 21 day or panicle differentiation stages came from the applied fertilizer. The N which accumulated after these stages came from native soil N, as it became available through pool substitution or mineralization. Guindo *et al.* (1994a) and Norman *et al.* (1992a) reported that the measured fertilizer N at maturity was significantly lower than at heading plus 21 days and panicle differentiation, respectively, but that was not seen in the present study (Fig. 1.3).

Total N accumulation in grain did not differ between cultural methods within a given year, but was higher in 1994 than 1993 or 1995 (Fig. 1.4). Total N accumulation in straw differed between DS and WS only at the four-leaf plus 14 day stage in 1994 and both the four-leaf plus 21 day stage in 1993 and 1994 (Fig. 1.4). Fertilizer N

accumulation in straw differed between cultural methods at numerous stages in 1994 and 1995 (Fig. 1.4), although the differences could be because of pool substitution. Fertilizer N accumulation in grain in 1994 and 1995 did not differ among cultural methods (Fig. 1.4). However, fertilizer N accumulation in grain was higher at maturity in DS than WS in 1993 (Fig. 1.4). Differences in grain N accumulation from year to year (Fig. 1.4) did not result in differences in grain dry matter (Fig. 1.1). This suggests that, given N sufficiency, and increase in plant N accumulation results in greater straw dry matter, but not greater grain dry matter (Fig. 1.1).

Remobilization of N from straw to grain occurred between 90% heading and maturity in 1994 and 1995. While straw dry matter increased during this period in 1994, total N accumulation in straw did not. This indicates that N loss was not due to a loss of plant material, but rather to movement to another plant part or out of the plant. During this period, total N accumulation in grain increased (Fig. 1.4). Since fertilizer N in the plant did not increase during this period (Fig. 1.3), N was supplied by native soil N and remobilized from straw. Fertilizer N in grain increased and fertilizer N in straw decreased by approximately the same amount between 90% heading and maturity (Fig. 1.4). This further indicates that straw N was the main source of grain N. Mae (1986) also reported that up to 70% of N in rice grain was remobilized from vegetative organs. Similarly, Hill (1980) concluded that N is remobilized from leaves to grain. He found that the pattern of remobilization changes little with a change in nutrient supply.

Drill-seeding and WS were equally efficient in preventing fertilizer N losses. There was no difference in fertilizer N recovery ((fertilizer N detected/fertilizer N applied)x100) by rice plants between cultural methods in 1994 and 1995 at maturity (Fig. 1.6). Likewise, there was no difference in fertilizer N recovery between cultural methods when residual fertilizer N in the soil was taken into account. The same was true in 1993, except in DS plants where more fertilizer N was taken up after 90% heading.

There were few differences in dry matter, root length density, N accumulation, or fertilizer N recovery by the rice plant between DS and WS any year. When differences did occur, they generally occurred early to mid-season, particularly at the four-leaf plus 21 day stage. This is related to the timing of N fertilization. In WS, N was incorporated before flooding and seeding, so more N was available to plants early in the growing season. Plants had a limited root system at these stages (Fig. 1.2), so were not able to take up most of the available N. In DS, N was not applied until the four-leaf stage, so plants needed to rely on native N for their growth very early in the season. In addition, differences in root growth between 1993 and 1994 affected dry matter and N accumulation under each cultural method. After the four-leaf plus 21 day stage, DS and WS plants did not differ in the factors measured, with the exception of dry matter at the heading plus 21 day stage in 1994 and 1995 (Fig. 1.1). At maturity, however, there were no differences between cultural methods.

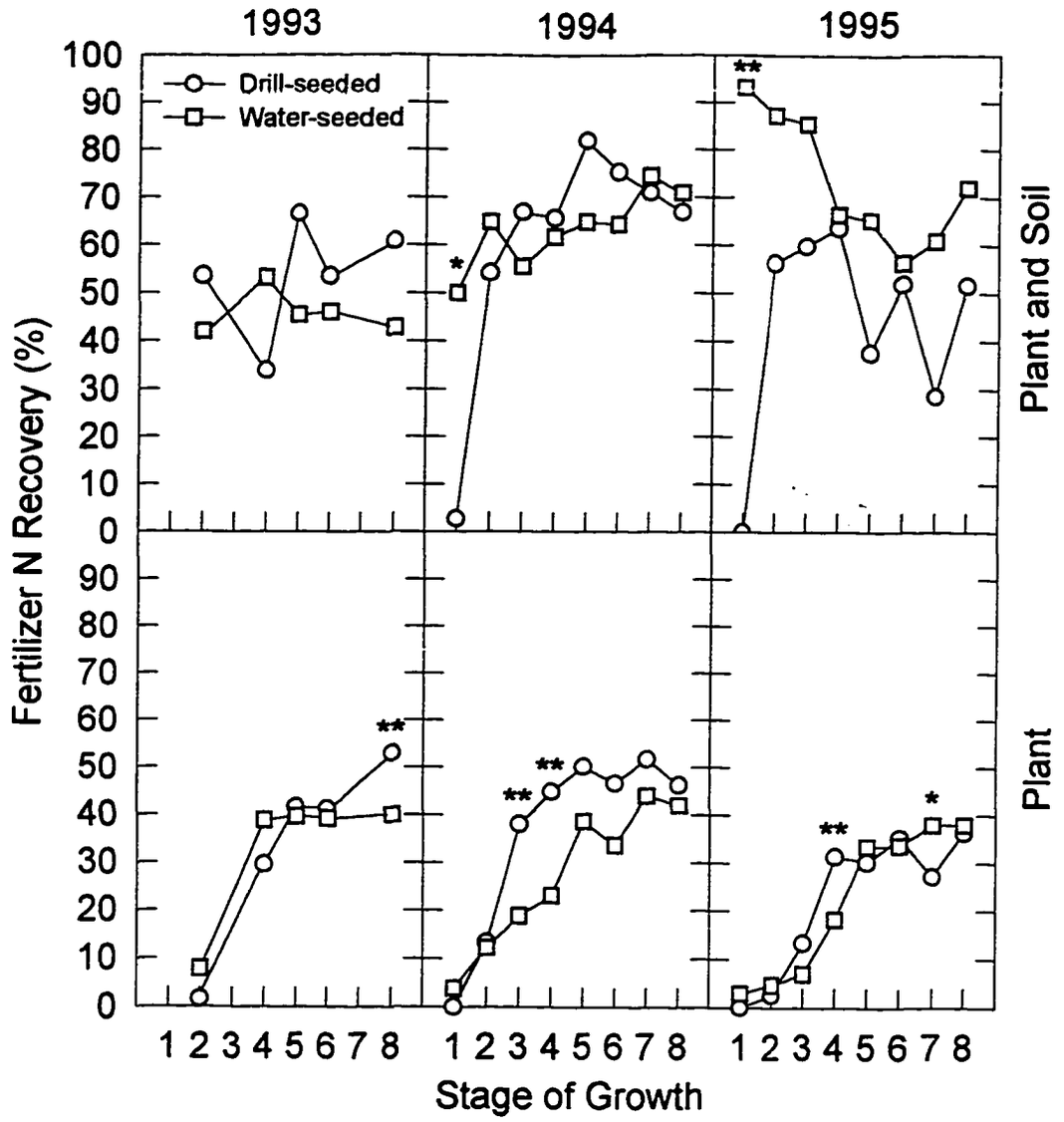

Fig. 1.6. Recovery of applied fertilizer nitrogen (N) in drill-seeded (DS) and water-seeded (WS) rice culture. Stages of growth are: 1=four leaf (4L), 2=4L + 7 days, 3=4L +14 days, 4= 4L + 21 days, 5=panicle differentiation, 6=90% heading, 7=90% heading + 21 days, 8=maturity. Asterisks indicate significant differences between DS and WS at α=.05 (*) or α=.01 (**).

Temperature Effects

Total dry matter (Fig. 1.1) and total N accumulation (Fig. 1.3) were greater in 1994 than 1993 or 1995, but grain yields did not differ between years (Fig. 1.1). Because grain yields did not differ, total dry matter differences were a function of differences in straw dry matter each year. Growing conditions were similar during the three years, however, average maximum air temperatures were significantly higher in 1994 than 1993 during the last two weeks of April, and lower in 1994 than 1993 or 1995 during the last two weeks of August (Fig. 1.7). The crop headed during the last two weeks of July and matured during August each year. Kuroyanagi and Paulsen (1985) reported that at daytime temperatures over 30°C, the dry weight of rice shoots four weeks after anthesis was less than at lower temperatures. Mikkelsen and De Datta (1991) and Nishiyama (1976) reported that shoot growth increased linearly from 18-33°C, and above that range, growth decreased. Maximum air temperatures throughout 1994 were within the range where there was no high-temperature reduction of straw dry matter (Fig. 1.7), and straw dry matter increased until heading plus 21 days (Fig. 1.1). The slight decrease in straw dry matter from heading plus 21 days until maturity can be explained by leaf senescence. Temperatures in 1993 and 1995 were frequently higher than 33°C (Fig. 1.7). In 1993, the high temperatures occurred immediately after 90% heading, explaining the decrease in rate (slope) of straw dry matter between 90% heading and maturity (Fig. 1.1). In 1995, the high temperatures occurred around panicle differentiation and again between 90% heading and maturity (Fig. 1.7), explaining the low rate of increase in dry matter beginning at panicle differentiation,

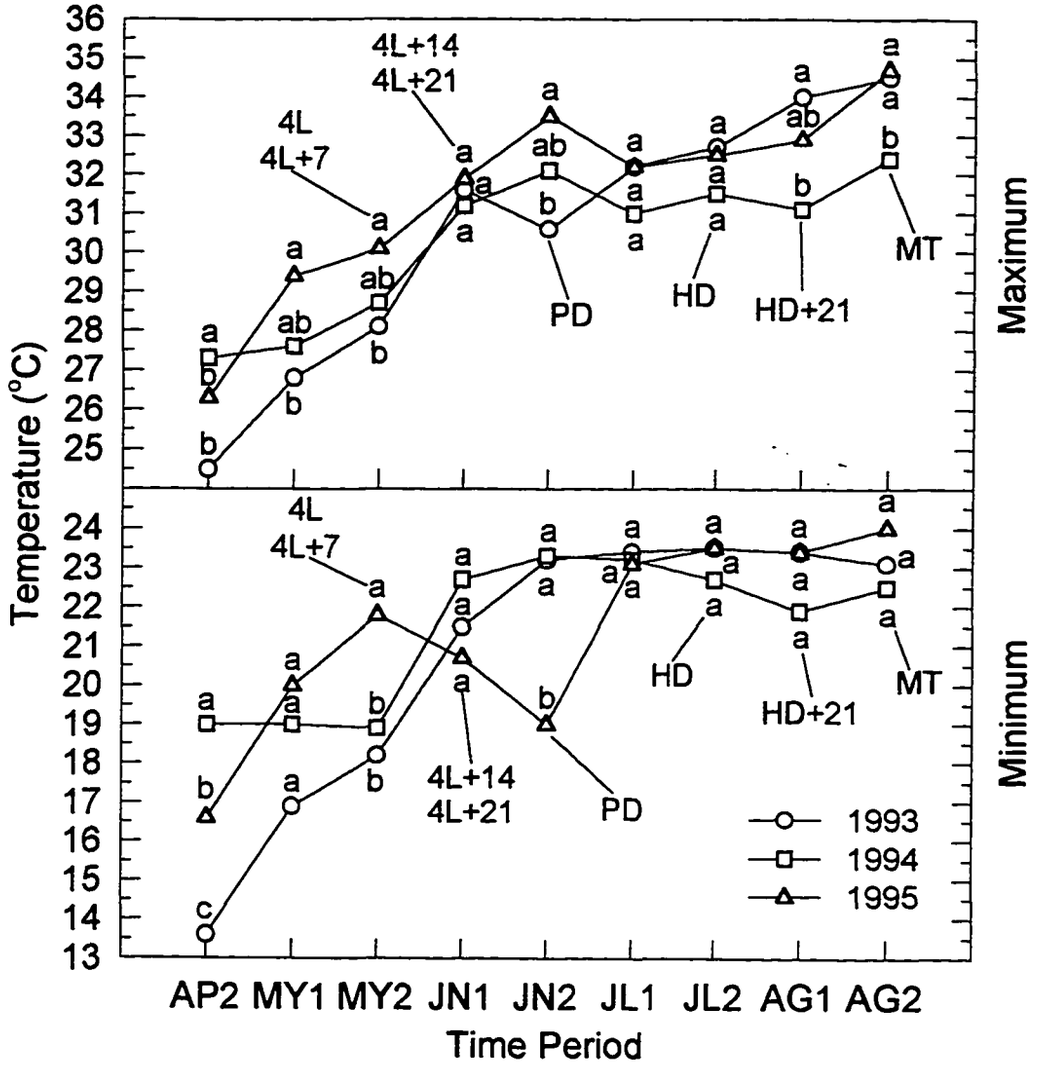

Fig. 1.7. Means of maximum and minimum air temperature for 15- or 16-day periods from April 16 to August 31 in 1993, 1994, and 1995. Time periods are: AP2=April 16-30, MY1=May 1-15, MY2=May 16-31, JN1=June 1-15, JN2=June 16-30, JL1=July 1-15, JL2=July 16-31, AG1=August 1-15, AG2=August 16-31. Stages of growth are: 4L=four leaf, 4L+7=4L + 7 days, 4L+14=4L plus 14 days, 4L+21=4L plus 21 days, PD=panicle differentiation, HD=90% heading, HD+21=HD plus 21 days, and MT=maturity. Years at the same time period with a common letter are not significantly different (α=.05).

and ultimately, the lowest dry matter yield in any of the three years (Fig. 1.1). Differences in surface root growth (Fig. 1.2) might be related to temperature. Although soil temperature data are not available, it stands to reason that greater air temperature should result in greater soil temperature. It is likely that the penetration of radiant energy and heat energy from air above the soil surface would decrease with increasing soil depth. Therefore, the warming effect would affect the surface soil more than the subsurface soil. A comparison of surface root growth with air temperature suggests that higher temperature might favor root growth. In 1995 (Fig. 1.7), the maximum temperature was very high (33.5°C) at panicle differentiation (which occurred during the latter 2 weeks of June), and at this stage of development, surface root growth increased dramatically (Fig. 1.2). After that, the temperature decreased and then increased again, and surface root growth followed roughly the same pattern. In 1993, the maximum temperature was above 30°C from panicle differentiation to maturity (Fig. 1.7) and root growth increased from panicle differentiation to maturity (Fig. 1.2). In 1994, the temperature was lower than either 1993 or 1995 (Fig. 1.7), and surface root growth was less than either year (Fig. 1.2). Subsurface root growth did not differ between years, and it is possible that subsurface soil temperatures did not differ either. It is important to state that this relationship between temperature and root growth is derived from air temperature and root growth data, and would be strengthened with soil temperature data and controlled conditions. There is no mention in the literature of a relationship between temperature and rice root growth. Kovar *et al.* (1992) found no correlation between root length density and soil temperature in

corn root growth. Research on soil temperature influences on corn root growth indicates that growth became more vertical as the soil temperature increased from 17 or 18°C to 30 or 36°C (Mosher and Miller, 1972; Onderdonk and Ketcheson, 1973). If this were true for rice roots, one would expect an increase in subsurface root growth, but that did not occur. The relationship between rice root growth and temperature could be an area for further study.

There is little research on temperature effects on N uptake by rice plants. Ta and Ohira (1982) found that N uptake increased through the temperature ranges in their study (9-30°C), although the rate of increase was less from 24-30°C than from 9-24°C. However, N mineralization appears to benefit from higher temperatures, at least up to 40°C (Kenney and Sahrawat, 1986; Reddy and Patrick, 1983). This suggests that in 1993 and 1995, when late season temperatures were higher than in 1994, there should have been more N mineralized and thus available, and more N absorbed by the rice plants than in other years. However, this was not the case (Fig. 1.3). It seems likely that N uptake is driven more by the demands of plant growth than the availability of soil N, except when soil N is a limiting factor. When conditions were better for plant growth, as in 1994, plants absorbed more N to meet those growth demands.

Despite differences in dry matter and N accumulation, grain yields were consistent from year to year (Fig. 1.1). Temperatures over 30°C at anthesis are known to increase sterility among some rice cultivars (Yoshida *et al.*, 1981). Tashiro and Wardlaw (1991) reported that temperatures over 30°C resulted in a decrease in grain dry weight, however, if the higher temperatures occurred later than 18 days after

heading (early August in Fig. 1.7), they found no reduction in grain dry weight. Temperatures above 30°C occurred from the four-leaf plus 21 day stage each year in the current experiment, but the highest temperatures did not occur until well after heading (Fig. 1.7). It appears that temperature did not affect the grain yield in this experiment, and that might be due to the late stage in which very high temperatures occurred.

Average minimum air temperatures do not appear to affect plant growth or N accumulation. Within the range of minimum temperatures in the present study (Fig. 1.7), there is no indication in the literature of detrimental effects on the rice plant, nor is there any suggestion that a daily vernalization is needed to sustain optimum plant metabolism. Furthermore, when fluctuations in plant growth and N accumulation were evidenced (between panicle differentiation and maturity), the minimum temperatures did not differ significantly (Fig. 1.7, with the exception of panicle differentiation in 1995), whereas the maximum temperatures did (Fig. 1.7).

Conclusions

Nitrogen accumulation before panicle differentiation was greater than that following panicle differentiation, although total N accumulation differed from year to year. Late season N demand by ripening grain was satisfied by remobilization of N from straw to grain. Total dry matter of rice plants increased throughout each season, with the greatest increases occurring after panicle differentiation. Grain dry matter at harvest did not differ from year to year. Early N applications are very beneficial, as

they encourage root and shoot growth, which will be a major source of N for ripening grain later in the season.

Fertilizer N accumulation differed from year to year, and the differences were not consistent between DS and WS. This suggests that multiple years of data are needed to improve the accuracy of field measurement with labeled fertilizer.

There were no differences in any factor measured between the heading plus 21 day and maturity stages in either DS or WS any year. This suggests that experimental plots harvested at either stage would provide interchangeable data and would allow researchers some freedom in planning harvests within the constraints imposed by available labor or weather.

Air temperatures during the last part of the growing season were higher than the optimum range in 1993 and 1995 but not in 1994. Straw dry matter and N accumulation were reduced, and surface root length density was increased, when the temperature exceeded 33°C. Grain yield was not affected by the temperatures seen in the current study. It appears that grain yield is controlled more by genotype than by the availability of N or appropriate temperature, provided that a sufficient supply of N is available.

Drill-seeded and water-seeded culture did not differ at early and late stages of development in any year in any factor measured. Mid-season differences are attributable to the timing of the N application and variations in root growth. This suggests that under Louisiana conditions data from experiments in one cultural system

can be generalized to conditions in the other system, particularly with respect to the mature plant.

CHAPTER 2

RICE PLANT GROWTH AND NITROGEN ACCUMULATION FROM A MIDSEASON APPLICATION

Introduction

Nitrogen fertilization in rice is extensive throughout the world, but fertilizer N recovery is seldom more than 30-40% under normal conditions, and 60-65% under optimum conditions (De Datta, 1981). In order to improve fertilizer N efficiency, split fertilizer applications which coincide with plant demand have been used (Mengel and Wilson, 1988; Wells and Johnson 1970).

Results of studies on the effectiveness of split applications often do not agree. Reddy and Patrick (1976) reported no difference in grain and straw yields between preflood applications of N as ammonium sulfate and a preflood-midseason split application when the midseason N was applied before PI. However, they saw a reduction in grain yield when the topdress was applied at PI. They did show that the split application resulted in greater total N in the grain. In a second study (Reddy and Patrick, 1978), grain yields did not vary between preflood N and a preflood-midseason split application when ammonium sulfate was the N source, but the preflood-midseason split application had greater grain yields when urea was the N source. This study also showed greater N accumulation in grain and straw when a split application was applied. Westcott *et al.* (1986) reported that there were no differences in straw or grain yields between preplant or preflood N and split applications at PI. Recovery of fertilizer N was greater with split applications than with preplant or preflood N. Norman *et al.*

(1990) found that a three-way split N application generally did not affect grain yields. Results from Wells *et al.* (1989) were as varied as the locations of the experiments in Arkansas, California, Louisiana, Mississippi, and Texas. A three-way split application frequently resulted in greater N efficiency and grain yields than preplant or preflood N applications. Norman *et al.* (1989) found greater grain yields and N accumulation with a three-way split N application at PD and PD plus 10 days than with only an early season application. Bollich *et al.* (1991b, 1993a) reported no difference in grain yield between a single application or a two-way split, but a significant yield reduction in a three-way split when the third application was at PD. However, in 1992 they found that a two-way split application resulted in lower grain yields than a single application, and a three-way split application resulted in lower grain yields than a two-way split application (Bollich *et al.*, 1994a).

No clear conclusions can be draw from these studies. The implication is that a preplant- or preflood-midseason split application of N generally results in greater N accumulation, but not necessarily an increase in grain yield than when all N is applied early. Under certain conditions, the increase in N accumulation does result in an increase in grain yield. There was generally no decrease in grain yield due to split N applications, except when part of the midseason application was at PD or later.

Little information is available on plant N accumulation after a midseason application. In an experiment comparing seeding methods and split applications of N, De Datta *et al.* (1988) reported differences in the pattern of N accumulation by the plant at high N rates, but no difference at low N rates. Wilson *et al.* (1989) examined

N accumulation by the plant from a three-way split application. Their results showed that most of the N from a midseason application was recovered within 3 days after application. Except for their early season results, recovery appeared to increase and then decrease over time, rather than following a linear or curvilinear pattern.

The purpose of this study was to measure plant dry matter, root length density, and N accumulation after a midseason N application, and to determine the length of time during which midseason N is accumulated by the plant.

Materials and Methods

Field experiments were conducted in 1993 and 1995 at the Rice Research Station in Crowley, LA, on a Crowley silt loam soil. Soil chemical and physical properties are summarized in Table 1.1. 'Cypress' rice was drill-seeded in 17.8 cm rows at 112 kg ha^{-1}. Urea was surface applied at 101 kg N ha^{-1} at the four-leaf stage of plant growth. The field was flooded, and the flood was maintained throughout the experiment. Prior to PI, retainers were randomly distributed throughout the field. In 1993, retainers constructed from polyvinyl chloride pipe measuring 61 cm in diameter and 30 cm in height were used. In 1995, retainers constructed from stainless steel measuring 75 cm square and 30 cm in height were used. Retainers were inserted into the soil to a depth of 10 cm, creating 15 microplots. At PI, a topdress of ^{15}N-labeled urea (2.39 atom% ^{15}N in 1993, 2.09 atom% ^{15}N in 1995) was broadcast into the floodwater in each microplot at 67 kg N ha^{-1}. Unlabeled urea was broadcast into the floodwater surrounding the microplots at the same N rate.

Microplots were harvested at 1 day after topdress (DAT), 3 DAT, 7 DAT, 14 DAT, and at 90% heading (35 DAT). All above ground plant material in three randomly selected microplots was harvested by hand at each sampling date. In addition to plant material, soil samples were collected with a 1.8 cm diameter hand probe to a depth of 15 cm. Two cores were collected from each plot in 1993, and four cores in 1995. Root samples were also collected with the hand probe to a depth of 22.5 cm. Four cores were collected from each plot and separated into 0- to 7.5-cm, 7.5- to 15-cm, and 15- to 22.5-cm depth increments.

Plants were oven-dried at 60°C to a constant weight, while soil samples were air dried. Samples were ground in a Wiley mill to pass a 1 mm mesh sieve. Subsamples of dried plant tissue and soils were digested in concentrated sulfuric acid. The semi-micro Kjeldahl procedure was followed for total N determination (Bremner and Mulvaney, 1982). Ethanol (95%) was distilled between samples to prevent ^{15}N cross-contamination during distillation. Distillates were acidified with 2 mL of .08*N* acid and evaporated to dryness for ^{15}N analysis (Hauck, 1982). Nitrogen atom% was determined on a Du Pont 21-621 mass spectrometer. Roots were separated from soil with a root washing system. Root length was determined with an edge discrimination technique developed by Pan and Bolton (1991), and root length densities were calculated for each sample.

Data were analyzed with the SAS System (SAS Institute, 1994). Analysis of variance with the GLM procedure was used to determine if significant differences existed among the harvest dates. Comparisons of means of each harvest date and the

90% heading sample (the control) were made with Dunnett's test (α=.05). Assumptions for the validity of the analyses were checked.

Results and Discussion

Most midseason N was accumulated within 7 days after application. Accumulation of midseason N began immediately after application, but leveled off after 7 DAT (Fig. 2.1). Each harvest date is compared to the 90% heading sample (35 DAT). By 90% heading, fluctuations resulting from midseason N application had long ceased, allowing that sampling date to be considered a control for maximum midseason N accumulation. At 1 and 3 DAT, midseason N accumulation was still significantly different from the 90% heading sample. However, by 7 DAT, N accumulation had increased to where there was no difference from the 90% heading sample. This shows that from 7 DAT, there was no significant increase in midseason N accumulation throughout the remainder of the experiment, and that most of the midseason N was accumulated within 7 days after application. Other researchers also found that accumulation of midseason N occurred within a few days of application. Wilson *et al.* (1989) reported that most midseason N was accumulated by 3 days after application. They found no statistical difference between this stage and harvests up to 14 days after midseason N was applied. Brandon and Wells (1986) reported that accumulation of midseason N occurred within 4 days of application. In the current study, N accumulation continued 3 to 4 days longer than that reported in these previous studies.

Approximately half of the midseason N was not accumulated by the crop. This is approximately the same recovery rate as when all N is applied preflood (Chapter 1).

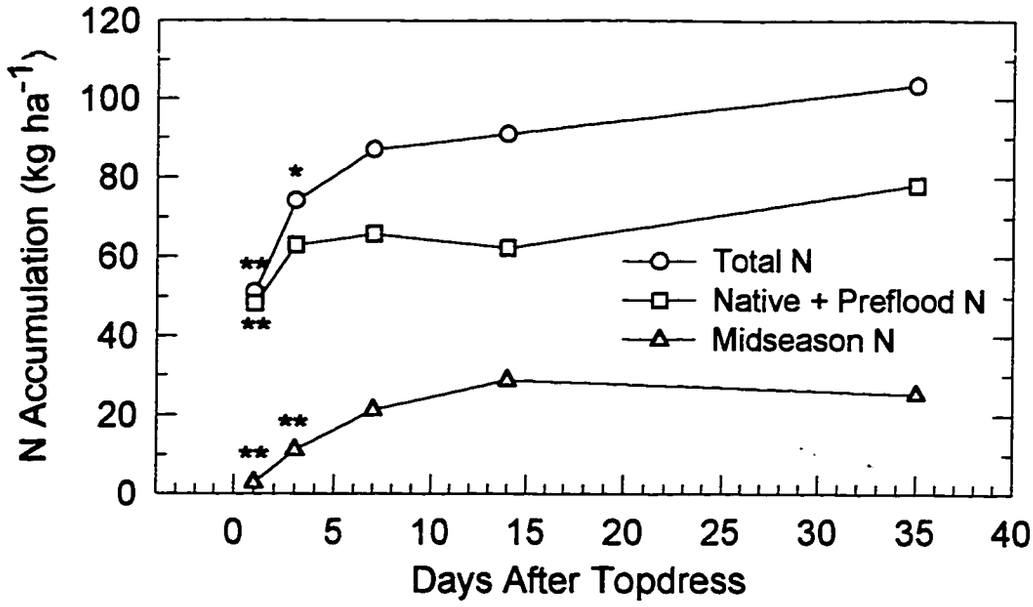

Fig. 2.1. Nitrogen (N) accumulation by rice plants after a midseason N topdress in 1993 and 1995. Total N includes preflood and midseason fertilizer N, and native soil N. Asterisks above points indicate significant differences between that point and the 90% heading sample (35 days after topdress) at α=.05 (*) or α=.01 (**).

Because midseason N was broadcast into the floodwater, ammonia volatilization can be expected to have been the source of much of the N loss (Fillery and Vlek, 1986). However, because root growth, and particularly surface root growth, was extensive at PI, it is not surprising that much of the midseason N was accumulated by the plant. Root length density did not differ significantly at the surface (0-7.5 cm depth) between the time of midseason N application and 90% heading (Fig. 2.2), suggesting either that maximum root length density was achieved by panicle initiation, or that a midseason N application had no effect on further root growth. Beyrouty *et al.* (1988, 1996) reported that maximum root length of rice occurs between PI and booting. There was a sufficient mass of roots at the soil surface at PI able to absorb about half of the applied N before volatilization losses could occur. This phenomenon was reported earlier by Brandon and Wells (1986). N accumulation continued throughout the experiment. Most of the N accumulated in the first 14 DAT came from the midseason application (Fig. 2.1). However, the N that accumulated after came from native and preflood N. Data in Chapter 1 showed that accumulation of preflood N was completed by panicle differentiation (about 14 DAT in the current experiment). Therefore, native soil N was the N source for late season N accumulation in this study.

An additional N topdress at 7 DAT would provide more available N for the plant, however, this topdress would come from a reduction in preflood N or midseason N rates, or an increase in the total N applied. If preflood N or midseason N rates were reduced, it is unlikely that the same level of N accumulation would be realized by 7 or 14 DAT as in this study, suggesting that there would be no benefit to a three-way split

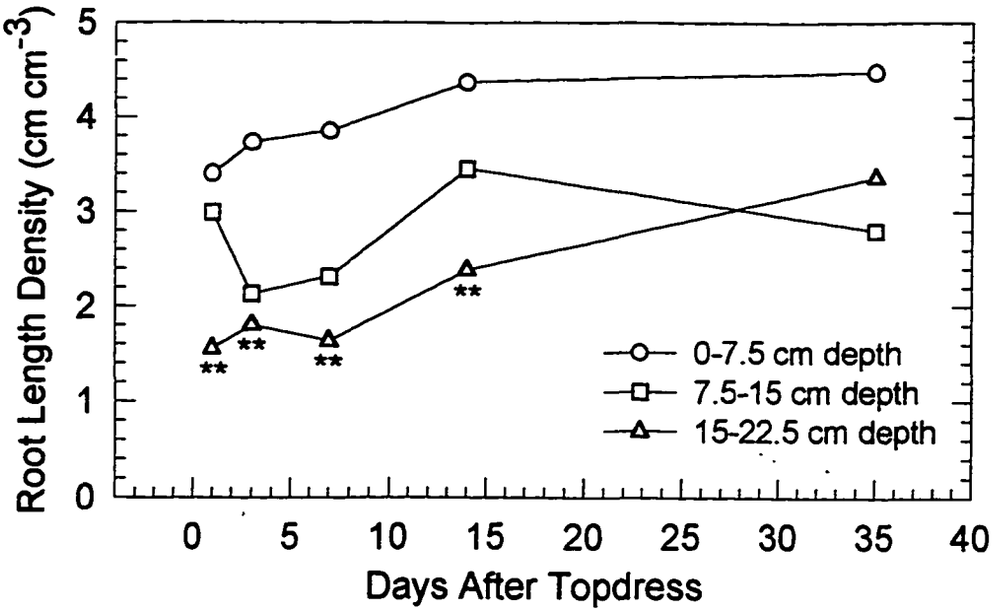

Fig. 2.2. Root growth of rice plants at three depth increments after a midseason N topdress in 1993 and 1995. Asterisks above points indicate significant differences between that point and the 90% heading sample (35 days after topdress) at α=.05 (*) or α=.01 (**).

application. Bollich *et al.* (1994a) showed that a three-way split N application did not result in greater N accumulation. Furthermore, a three-way split application with a topdress at PD resulted in a reduction in grain yield (Bollich *et al*, 1991b, 1993a).

Surface (0-7.5 cm depth) and deep (15-22.5 cm depth) root length density increased from PI to 90% heading (Fig. 2.2). Subsurface (7.5-15 cm depth) root length density decreased immediately after PI for about 7 days, then increased during the next 7 days (Fig. 2.2). At PI, the rice plant began a shift from vegetative to reproductive growth. It is possible that at this stage, depletion of root reserves began in order to provide energy and materials for reproductive growth, explaining the initial decline in root length density in subsurface roots. However, with an influx of N from the midseason application, new root growth might have been stimulated, offsetting root losses at the surface, and explaining the subsequent increase in root length density in subsurface roots. Deep root growth appears to have increased the most and at 90% heading, it was significantly greater than at any previous stage. Surface and subsurface root growth was probably sufficient for mining the available nutrients at these depths. Deep root growth occurred as the season progressed, since nutrient demands increased, and nutrients in the upper soil layers were depleted. This is particularly true with regard to N. It was shown earlier that accumulation of N applied at midseason was complete by 7 DAT, however, total N accumulation continued to increase (although not significantly) until 90% heading (Fig. 2.1). Therefore, by 14 DAT, there should have been little N available from what was applied preflood or midseason. This suggests that N accumulated after 14 DAT had to come from native soil N that was

being mineralized during the growing season. Because this mineralization is slow under flooded conditions, the plant needed to explore a larger area to take up sufficient N to accommodate the needs of reproductive growth. Because of competition from adjacent plants, it is possible that increases had to take place downward, explaining the increased deep root growth.

Dry matter does not appear to have been affected by the midseason N application in 1993 or 1995. Dry matter increased from the time of the midseason application until 90% heading without any change in pattern corresponding to the midseason application (Fig. 2.3). Analysis of variance of plant dry matter data (Table 2.1) showed interaction between year and harvest date. Therefore, each year was analyzed separately, and means are presented for each year. The increase in dry matter in this experiment is approximately the same as the increase in dry matter during the same stages of growth reported in Chapter 1, where all N was applied preflood. The increase in dry matter appears to be linear, suggesting that plant growth, at least within satisfactory levels of N, is more a function of genetics than N level. Increases in N level, initially or after a midseason N application, simply change the N concentration of the plant material.

Conclusions

A split application of N at preflood and midseason resulted in accumulation of the midseason N within 7 days after application. However, only about half of the applied N fertilizer was recovered. This recovery rate was not different from a single preflood application, suggesting that there would be no advantage to a split application

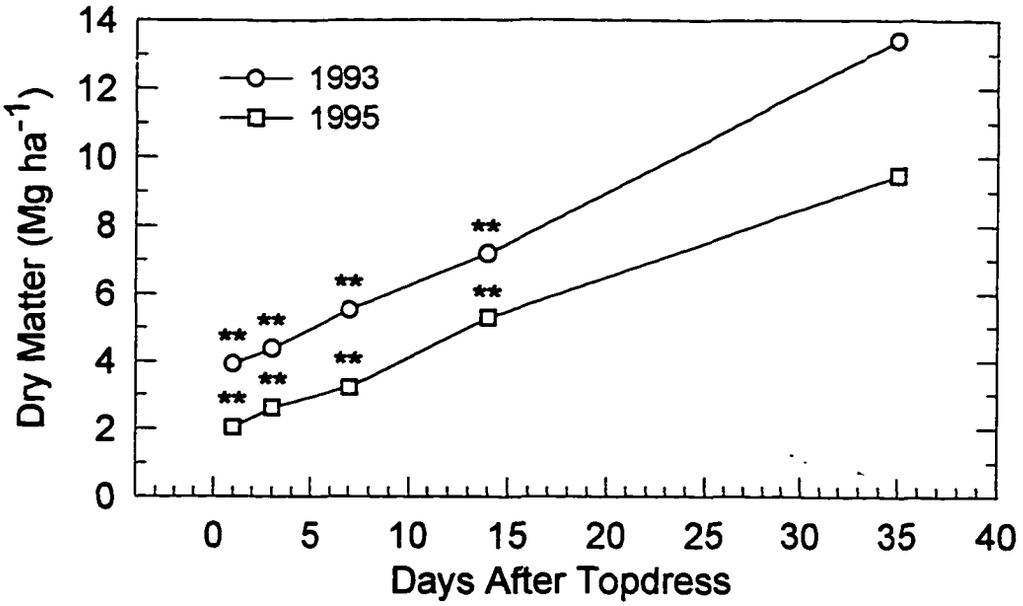

Fig. 2.3. Total dry matter of all above-ground plant material of rice plants after a midseason N topdress. Asterisks above points indicate significant differences between that point and the 90% heading sample (35 days after topdress) at α=.01 (**).

Table 2.1. Analysis of variance of the effects of year and harvest date on plant growth and nitrogen accumulation.

Effects	Plant Dry Matter			Total N	Mid-season N	Root Length Density: Profile Depth (cm) 0-7.5	7.5-15	15-22.5
	93&95	93	95	93&95	93&95	93&95	93&95	93&95
Year	**			**	ns	*	ns	ns
Harvest date	**	**	**	**	**	ns	ns	**
Year x Harvest date	*			ns	ns	ns	ns	ns
C.V. (%)	11.4	11.9	9.5	20.4	21.3	21.6	29.1	18.0

Significance of F-values: *=p≤.05; **=p≤.01; ns=not significant.

of fertilizer N to flooded rice. However, since a split application requires a second field application, generally by airplane, a single preflood application of N should be economically advantageous. Because the focus of the present study was on N accumulation, grain yields were not considered. However, the literature indicates that differences in grain yield between a single preflood application and a split application of N generally do not occur. This reinforces preference for a single early N application over split applications.

CHAPTER 3

RICE VARIETY DIFFERENCES IN DRY MATTER AND NITROGEN ACCUMULATION AS RELATED TO PLANT STATURE AND MATURITY GROUP

Introduction

Rice is one of the major food crops in the world today (Fageria *et al.*, 1991), providing the primary source of calories for 40% of the world's population (De Datta, 1981). Because of its extensive cultivation, numerous varieties have been developed to meet the needs of different tastes, climates, cultural practices, and input levels.

Differences in N efficiency have been detected among rice varieties grown in Southeast Asia (De Datta, 1986) and in the United States of America (Bollich *et al.*, 1994b; Norman *et al.*, 1995). Brandon *et al.* (1985b) suggested that the amount and time of N application required for maximum yield differs among rice varieties. Some varieties require low N rates because of efficient N utilization or because lodging, disease, physiological problems, and other factors limit their yield at higher N rates. Other varieties require high N rates for maximum yield. Nowick and Hoffpauir (1984) noted that the level of N fertilization required for maximum yield appeared to differ between modern varieties and older varieties of rice. Many researchers have suggested that modern semi-dwarf varieties require higher N rates than taller varieties (Brandon and Wells, 1986; De Datta *et al.*, 1968; Guindo *et al.*, 1994b; Hartley and Milthorpe, 1982; Roberts, *et al.*, 1993; Tran Van Dat *et al.*, 1978; Wells and Turner, 1984), although others have found that N use was similar for both variety types (Norman, *et*

al., 1992b). Conclusions were frequently based on a comparison of one variety of each variety-type.

Little research has compared N use by variety-type or investigated mechanisms of N use that would differentiate varieties. However, methods for evaluating N efficiency in rice genotypes have been proposed. Broadbent *et al.* (1987) and De Datta and Broadbent (1988) suggested that genotypes be rated according to several plant parameters related to N use and efficiency. Using electron microscopy, Senanayake *et al.* (1991) found differences in growth among rice varieties, and suggested different N topdress strategies be used for early and late maturing varieties. Laza *et al.* (1993) found differences in chloroplast ultrastructure among rice varieties, and suggested that increases in chloroplast size were related to increases in chlorophyll, protein content, and dry weight. No research has related differences in N requirement to plant stature.

The objective of this study was to evaluate differences in dry matter and N accumulation among modern long-grain varieties in relation to plant stature (tall *vs.* semi-dwarf) and maturity group (early *vs.* very early). Modern varieties were compared to varieties no longer in production (old varieties). Comparisons were made between modern medium-grain varieties as well as old and modern long-grain varieties to determine differences within modern varieties.

Materials and Methods

Greenhouse Experiment

Fifteen old and modern long-grain rice varieties (Table 3.1) were included in a 1994 greenhouse experiment. Pots 24 cm high were filled to the 15 cm mark with

Table 3.1: Characteristics of varieties used in the greenhouse and field experiments.

Variety-type	Characteristics			Variety (year and state of release)
	Stature	Maturity	Grain	
Old	very tall	late	medium	Carolina Gold (1694) Magnolia (1945 LA) Zenith (1936 AR)
Tall/early	tall	early	long	Katy (1989 AR) Kaybonnet (1994 AR) Newbonnet (1983 AR)
Tall/very early	tall	very early	long	Adair (1992 AR) Jackson (MS) Tebonnet (1984 AR)
Semi-dwarf/early	semi-dwarf	early	long	Cypress (1992 LA) Lacassine (1991 LA) Lemont (1983 TX)
Semi-dwarf/very early	semi-dwarf	very early	long	Jodon (1994 LA) Maybelle[1] (1989 TX) RT 7015 (TX)
Medium-grain	semi-dwarf tall	early early	medium medium	Bengal (1992 LA) Mars (1977 AR) Orion (1991 AR)

[1]Short-statured but does not contain the semi-dwarf gene.

Crowley silt loam soil. Initial soil chemical properties were: pH, 5.8; organic matter, 1.26%; extractable P, 24 mg kg^{-1}; exchangeable Na, 70 mg kg^{-1}; exchangeable K, 57 mg kg^{-1}; exchangeable Ca, 1065 mg kg^{-1}; exchangeable Mg, 173 mg kg^{-1}; extractable Zn, 2.24 mg kg^{-1}; extractable S, 16.37 mg kg^{-1}; extractable As, 11.51 mg kg^{-1}; and extractable Fe, 122 mg kg^{-1}. Urea was applied at the rate of 1.35 g urea per pot (190 kg N ha^{-1}) and incorporated in the top 3 cm of soil. Pots were flooded. Approximately 25 seeds of a variety were water-seeded into each pot. Supplementary artificial lighting was used to provide a 16-hour day. The experimental design was a randomized complete block with three replications. Plants were rerandomized within each block twice weekly until 21 days after sowing, and weekly thereafter. Treatments were thinned to 15 plants pot^{-1} 14 days after sowing and to 5 plants pot^{-1} 21 days after sowing. As plant height increased, the depth of floodwater was increased to a maximum of 8 cm. A 0.1*N* copper sulfate solution was sprayed into the floodwater periodically to control algae. Pots were drained 50 days after sowing.

Plants were harvested 52 days after sowing, oven-dried at 60°C to a constant weight, and ground in a Wiley mill to pass a 1 mm mesh sieve. Subsamples of plant material were digested in concentrated sulfuric acid. The semi-micro Kjeldahl procedure was followed for total N determination (Bremner and Mulvaney, 1982).

Field Experiment

An experiment was conducted in 1995 with flooded rice at the Rice Research Station in Crowley, LA, on a Crowley silt loam soil. Initial soil chemical properties were: pH, 6.5; organic matter, 1.13%; extractable P, 78 mg kg^{-1}; exchangeable Na, 98

mg kg^{-1}; exchangeable K, 66 mg kg^{-1}; exchangeable Ca, 1240 mg kg^{-1}; exchangeable Mg, 201 mg kg^{-1}; extractable Zn, 7.45 mg kg^{-1}; extractable S, 9.09 mg kg^{-1}; extractable As, 0.71 mg kg^{-1}; and extractable Fe, 183 mg kg^{-1}. The experimental design was a randomized complete block with four replications. Treatments were a factorial arrangement of three preflood N levels (0, 67, and 135 kg N ha^{-1}) and six variety-types (old varieties, modern medium-grain varieties, and four groups of modern long-grain varieties). The modern long-grain varieties were arranged as a factorial combination of two statures (tall and semi-dwarf) and two maturity groups (early and very early). Three varieties were nested within each variety-type. Varieties and their characteristics are summarized in Table 3.1. Rice was drill-seeded in 17.8 cm rows at 112 kg seed ha^{-1}. Field plots measured 2.1 x 7.6 m, and one replicate of one variety at one N level was planted in each plot. Prior to permanent flooding at the four-leaf stage of plant growth, urea was broadcast on the soil surface. The permanent flood was maintained until 14 days before harvest.

All above-ground plant material from three randomly selected 30 x 30 cm squares within each plot was harvested by hand 49 days after sowing (early harvest) and 25 days after 50% heading (late harvest). Plant material was oven-dried at 60°C to a constant weight, and the late harvest was separated into grain and straw. Plant material was ground in a Wiley mill to pass a 1 mm mesh sieve, and subsamples were analyzed for total N determination on a Leco FP-428 (Leco Corp., St. Joseph, MI).

Statistical Analysis

Data were analyzed with the SAS System (SAS Institute, 1994). Analysis of variance with the GLM procedure was used to determine if significant differences existed among N levels, variety-types, and varieties. Other comparisons were made with contrasts of marginal means and Dunnett's test. Assumptions for the validity of the analyses were checked.

Results and Discussion

Greenhouse Experiment

Total N accumulation did not vary with variety-type (Table 3.2). Although the variety-types are known to differ in growth habit, there was no evidence that growth habit had an effect on N accumulation. The data suggests that different variety-types did not differ in their ability to take up and store N, and that they might not differ in their N requirement. Furthermore, varieties of a particular variety-type did not exhibit any variability (Table 3.2), suggesting that varieties were uniform in their N accumulating ability.

Dry matter production differed between old varieties and long-grain varieties (Table 3.2), with old varieties producing much more dry matter than the others (Table 3.3). This suggests that the difference between rice varieties is not related to stature or earliness, but rather is between old and modern varieties. Breeding programs for modern varieties have attempted to produce varieties that have higher harvest indices (grain yield/total yield) so that resources can be used more efficiently in grain production. The data show that modern varieties accumulate as much N as old

Table 3.2: Analysis of variance of the main effects, and significance of contrasts in the greenhouse experiment. Variety-types considered were old and modern long-grain varieties.

	Dry Matter, Total	N Accumulation, Total
Main effects:		
Type	**	ns
Variety(Type)	**	ns
Contrasts:		
Type:		
old *vs.* all others	**	
early *vs.* very early	ns	
semi-dwarf *vs.* tall	ns	
stature x maturity	ns	
Variety(Type):		
semi-dwarf/early	*	
semi-dwarf/very early	**	
old	**	
tall/early	ns	
tall/very early	ns	
C.V. (%)	14.8	14.4

Significance of F-values: *p≤.05; **p≤.01; ns-not significant.

Table 3.3: Means of groups of varieties grown in the greenhouse contrasted to one another. Variety-types considered were old and modern long-grain varieties. Pairs of means in parentheses are not significantly different from each other at α=.05.

Contrast	Dry Matter, Total	N Accumulation, Total
Old *vs.* all others	4.60, 3.34	(64.7, 62.9)
Early *vs.* very early	(3.21, 3.46)	(62.7, 63.2)
Semi-dwarf *vs.* tall	(3.40, 3.28)	(63.9, 62.0)

varieties, but do not use that N in dry matter production. Modern varieties appear to efficiently store the accumulated N, presumably for later use in grain production (Chapter 1).

There was no evidence to suggest that semi-dwarf varieties have a higher N requirement than tall varieties, as has been proposed by many authors (Brandon and Wells, 1986; De Datta *et al.*, 1968; Guindo *et al.*, 1994b; Hartley and Milthorpe, 1982; Roberts *et al.*, 1993; Tran Van Dat *et al.*, 1978; Wells and Turner, 1984). Either a difference in dry matter production, or a difference in N accumulation without a difference in dry matter production between semi-dwarf and tall varieties would indicate the possibility of a difference in N requirement. Neither was found (Tables 3.2 and 3.3). However, the crop in the greenhouse was not grown to maturity, and only one N level was used. Therefore, nothing conclusive can be determined about N requirement.

Varieties of each variety-type did not differ in total N accumulation, however, there were differences in dry matter production (Table 3.2). The three varieties of old and semi-dwarf variety-types differed in dry matter, suggesting that the varieties within these types were not uniform, whereas tall varieties were uniform.

The greenhouse experiment was a preliminary study to determine if there were differences in N accumulation and dry matter production among variety-types, and to determine if differences in the N requirement of semi-dwarf and tall varieties merited further study. Differences in N requirement between semi-dwarf and tall varieties were not suggested by the data. Furthermore, N accumulation was similar among all variety-

types. However, differences in dry matter were apparent between old and long-grain varieties, and old and semi-dwarf varieties of a variety-type were not uniform. Therefore, this greenhouse experiment suggested that further research in the field up to maturity was necessary to study differences in N accumulation and dry matter production among variety-types.

Field Experiment

Modern long-grain rice varieties are categorized by stature and maturity group. Height at maturity and the number of days required for 50% heading varied among variety-types (Tables 3.4 & 3.5a). For example, at 135 kg N ha^{-1}, very early varieties reached 50% heading 5 days sooner than early varieties, and semi-dwarf varieties were 16.9 cm shorter than tall varieties (Table 3.6). Varieties within a variety-type were not uniform in stature or maturity (Tables 3.4 & 3.5). However, each stature and maturity category represents a range of values, allowing for differences to be included within the same category. For all modern long-grain varieties (Table 3.7), no very early variety reached 50% heading after 87 days after planting, and no early variety reached 50% heading sooner than 87 days after planting. Likewise, no semi-dwarf variety was taller than 89.8 cm, and no tall variety was shorter than 93.1 cm. Although varieties within a variety-type were not identical, they were properly categorized, and therefore are appropriate for this study.

Varieties within each group were not randomly selected in this experiment, even though the objective of the experiment was to generalize about all varieties of each variety-type. In statistical terms, unless treatment levels are selected randomly,

Table 3.4: Analysis of variance of the main effects and interactions in the field experiment. Variety-types considered were old and modern long-grain varieties.

	Field, early		Field, late								Field	
	Dry Matter	N Accu.	Dry Matter			N Accumulation						
Effects	Total	Total	Straw	Grain	Total	Straw	Grain	Total	HI[1]	PE[2]	HD[3]	HT[4]
Level	**	**	**	**	**	**	**	**	**	**	**	**
Type	*	ns	**	**	**	**	**	ns	**	**	**	**
Variety(Type)	**	ns	**	**	**	ns	**	*	**	**	**	**
Level x Type	ns	ns	ns	*	ns	ns	**	ns	**	*	**	**
Level x Variety(Type)	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	**	ns
C.V. (%)	20.2	22.2	13.6	11.7	10.9	18.3	12.7	12.7	7.1	9.0	1.3	5.8

Significance of F-values: *p≤.05; **p≤.01; ns=not significant.
[1]Harvest index (grain yield / total yield).
[2]Physiological efficiency (grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}).
[3]Days to 50% heading.
[4]Plant height at maturity.

Table 3.5a: Significance of contrasts in the field experiment. Variety-types considered were old and modern long-grain varieties.

	Field, early		Field, late								Field	
	Dry Matter	N Accu.	Dry Matter			N Accumulation						
Contrast	Total	Total	Straw	Grain	Total	Straw	Grain	Total	HI[1]	PE[2]	HD[3]	HT[4]
Level:												
linear	**	**	**		**	**		**		.		
quadratic	**	*	**		**	ns		ns				
Type:												
old *vs.* all others	ns		**		**	**						
early *vs.* very early	ns		ns		ns	ns						
semi-dwarf *vs.* tall	**		**		**	**						
stature x maturity	ns		ns		ns	ns						
Variety(Type):												
semi-dwarf/early	*		ns	ns	ns		(ns)	ns	ns	ns	(**)	**
semi-dwarf/very early	**		ns	ns	ns		(ns)	ns	ns	ns	(**)	**
old	ns		ns	**	**		(**)	ns	**	**	(**)	**
tall/early	*		ns	**	ns		(**)	ns	**	**	(**)	ns
tall/very early	ns		**	**	**		(*)	*	*	ns	(**)	**

Significance of F-values: *p≤.05; **p≤.01; ns=not significant.
[1]Harvest index (grain yield / total yield).
[2]Physiological efficiency (grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}).
[3]Days to 50% heading.
[4]Plant height at maturity.

Table 3.5b: Significance of contrasts in the field experiment. Variety-types considered were old and modern long-grain varieties.

	Field, early		Field, late							Field	
	Dry Matter	N Accu.	Dry Matter			N Accumulation					
Contrast	Total	Total	Straw	Grain	Total	Straw	Grain	Total	HI[1]	PE[2]	HD[3]	HT[4]
Type x Level:												
at 0 kg N ha^{-1}:												
old *vs.* all others				**			ns		**	**	**	**
early *vs.* very early				ns			ns		**	**	**	*
semi-dwarf *vs.* tall				ns			ns		**	ns	*	**
at 67 kg N ha^{-1}:												
old *vs.* all others				**			**		**	**	**	**
early *vs.* very early				*			ns		**	ns	**	ns
semi-dwarf *vs.* tall				ns			ns		**	*	**	**
at 135 kg N ha^{-1}:												
old *vs.* all others				**			**		**	**	**	**
early *vs.* very early				ns			ns		ns	ns	**	**
semi-dwarf *vs.* tall				*			**		**	ns	**	**

Significance of F-values: *p≤.05; **p≤.01; ns=not significant.
[1]Harvest index (grain yield / total yield).
[2]Physiological efficiency (grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}).
[3]Days to 50% heading.
[4]Plant height at maturity.

Table 3.6: Means of groups of varieties grown in the field contrasted to one another. Variety-types considered were old and modern long-grain varieties. Pairs of means in parentheses are not significantly different from each other at α=.05.

	Field, early	Field, late			Field	
	N Accumulation	N Accumulation			Days to 50%	Plant Height at
Contrast	Total	Straw	Grain	Total	Heading	Maturity
	kg ha^{-1}					cm
at 0 kg N ha^{-1}:						
old *vs.* all others	(17.1, 14.5)	31.4, 23.6	(33.1, 38.2)	(65.1, 61.8)	95, 84	98.0, 70.1
early *vs.* very early	(14.9, 14.0)	(25.7, 21.4)	(38.1, 38.3)	(63.8, 59.8)	88, 80	68.6, 71.7
semi-dwarf *vs.* tall	(13.8, 15.1)	(22.1, 25.1)	(38.7, 37.8)	(60.8, 62.9)	83, 84	68.2, 72.1
at 67 kg N ha^{-1}:						
old *vs.* all others	(61.2, 60.9)	54.9, 45.9	52.6, 67.7	(108.1, 113.5)	91, 82	113.3, 81.6
early *vs.* very early	(60.9, 61.0)	(45.2, 46.6)	(65.7, 69.6)	(110.9, 116.1)	85. 79	(81.2, 81.9)
semi-dwarf *vs.* tall	(61.4, 60.5)	42.7, 49.1	(69.5, 65.8)	(112.2, 114.8)	82, 83	75.9, 87.3
at 135 kg N ha^{-1}:						
old *vs.* all others	(95.6, 99.1)	80.5, 69.6	65.7, 86.4	146.3, 156.0	92, 85	127.0, 92.3
early *vs.* very early	(99.7, 98.5)	(70.2, 68.9)	(84.6, 88.2)	(154.9, 157.1)	88, 83	89.8, 94.9
semi-dwarf *vs.* tall	(95.1, 103.1)	(68.5, 70.6)	90.3, 82.6	(158.7, 153.2)	85, 86	83.9, 100.8

Table 3.7: The average length of time for modern long-grain varieties to attain 50% heading and the plant height of the varieties at maturity. Data from field experiment at 135 kg N ha^{-1}.

Variety-type	Variety	Days to 50% heading	Plant height at maturity
			cm
Tall/early	Katy	89	98.8
	Kaybonnet	87	99.5
	Newbonnet	90	94.9
Tall/very early	Adair	82	99.9
	Jackson	83	93.1
	Tebonnet	87	118.5
Semi-dwarf/early	Cypress	88	85.5
	Lacassine	87	82.3
	Lemont	90	77.7
Semi-dwarf/very early	Jodon	86	89.8
	Maybelle	74	83.6
	RT 7015	83	84.9

inferences can only be made about the particular levels selected, and not about the population in general (Neter *et al.*, 1990). However, for some of the variety-types in this experiment, few varieties adapted to the mid-South were available. Therefore, most of the varieties from the population of adapted varieties were included. Furthermore, there were several varieties of each type included in the experiment, *i.e.* there were six tall, six semi-dwarf, six early, and six very early varieties. If variability exists among tall or semi-dwarf varieties, or among early or very early varieties, it will likely be exhibited in the six varieties selected. For these reasons, it is possible to cautiously generalize about all varieties of a variety group, including those not tested.

The main effect of variety was significant in every factor except N accumulation in straw from the late harvest (Table 3.4). Additionally, there was an interaction between N level and variety in grain N accumulation and days to 50% heading (Table 3.4). Contrasting the varieties within each variety-type (Table 3.5a), dry matter and N accumulation late in the season were the same among semi-dwarf varieties, whether early- or very early-maturing. This indicates that the semi-dwarf varieties tested were very uniform, and suggests that this is true for other semi-dwarf varieties as well. Because of this uniformity, it is safe to conclude that other semi-dwarf varieties would have similar dry matter and N accumulation under the same conditions. Old and tall varieties did not exhibit the same uniformity among varieties. This indicates that the varieties in each of these variety-types respond to treatments differently, and therefore, responses seen in these particular varieties cannot be generalized to other varieties of the same type.

Among old varieties and the four groups of modern long-grain varieties, total N accumulation varied with N level (Table 3.4). However there was no interaction between N level and variety-type (Table 3.4). This indicates that N accumulation at a particular N level was the same for each of the 5 variety-types. Between 0 and 135 kg N ha^{-1}, N accumulation increased curvilinearly with N rate early in the field study, and linearly late in the field study (Table 3.5a). This suggests that the ability of the plant to absorb available N increased with plant age for all varieties, so that the effect of N rate on N accumulation increased as the season progressed. At N rates of 0, 67, and 135 kg ha^{-1}, average total N accumulation for all variety-types early was 15.0, 61.0, and 98.4 kg ha^{-1}, and late was 62.5, 112.4, and 154.0 kg ha^{-1}, respectively (Table 3.6).

Grain dry matter production varied with variety, variety type, and N rate (Table 3.4). At every N rate, modern long-grain varieties out-performed old varieties (Tables 3.5b & 3.8), suggesting that modern varieties produce higher yields than old varieties even at lower input levels. Varieties maturing very early had a higher grain yield than varieties maturing early only at 67 kg N ha^{-1}. In addition, grain dry matter of tall varieties increased less than that of semi-dwarf varieties with increasing N rate (Table 3.8). For both types, the increase in grain yield that accompanied each increase in N rate was less at higher N rates. However, it is not known whether grain yield would increase for semi-dwarf varieties and not tall varieties at a N rate higher than 135 kg ha^{-1}. Most modern varieties perform optimally in Louisiana at 135 kg N ha^{-1} (Bollich *et al.* 1994b, 1993b, 1992b, 1991a, 1990a; Brandon *et al.* 1985a), so that the contrast between the semi-dwarf and tall varieties at this N rate is particularly interesting. It

Table 3.8: Means of groups of varieties grown in the field contrasted to one another. Variety-types considered were old and modern long-grain varieties. Pairs of means in parentheses are not significantly different from each other at α=.05.

	Field, early	Field, late				
	Dry Matter	Dry Matter			Harvest	Physiological
Contrast	Total	Straw	Grain	Total	Index[1]	Efficiency[2]
	Mg ha^{-1}				ratio	
at 0 kg N ha^{-1}:						
old *vs.* all others	(.90, .87)	5.96, 3.77	3.06, 3.73	9.03, 7.50	.33, .50	46.2, 60.5
early *vs.* very early	(.89, .85)	(4.02, 3.51)	(3.69, 3.78)	(7.71, 7.29)	.48, .52	57.6, 63.3
semi-dwarf *vs.* tall	(.81, .93)	(3.48, 4.05)	(3.75, 3.72)	(7.24, 7.76)	.52, .48	(61.8, 59.1)
at 67 kg N ha^{-1}:						
old *vs.* all others	(2.87, 2.74)	10.57, 7.46	5.39, 6.54	(15.96, 13.99)	.34, .47	50.3, 57.9
early *vs.* very early	(2.75, 2.72)	(7:53, 7.39)	6.32, 6.75	(13.85, 14.12)	.46, .48	(57.1, 58.6)
semi-dwarf *vs.* tall	(2.68, 2.79)	6.78, 8.14	(6.63, 6.44)	13.41, 14.56	.50, .44	59.3, 56.4
at 135 kg N ha^{-1}:						
old *vs.* all others	(3.01, 3.12)	13.08, 10.29	6.19, 7.95	19.26, 18.23	.32, .44	42.4, 51.0
early *vs.* very early	(3.04, 3.20)	(10.29, 10.29)	(7.85, 8.04)	(18.14, 18.33)	(.43, .44)	(50.7, 51.3)
semi-dwarf *vs.* tall	2.87, 3.37	9.77, 10.81	8.14, 7.75	(17.91, 18.55)	.46, .42	(51.3, 50.6)

[1]Grain yield / total yield.

[2]Grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}.

suggests that the marketable yield of modern rice varieties is related to plant stature, and indicates that semi-dwarf varieties produce more grain than tall varieties. A similar contrast was not detected among maturity groups.

The mechanism for differences in grain yield is a difference in partitioning of dry matter between grain and straw. Nearly half the total dry matter in modern varieties was grain; this was not the case with old varieties (Table 3.8). The harvest index, a ratio of grain dry matter to total dry matter, of modern varieties was significantly higher than that of old varieties at every N level (Tables 3.5b & 3.8), showing that modern varieties partition more of their dry matter to grain than do old varieties. This accounts for the higher grain yields in modern varieties despite equal total N accumulation. Because of the greater portion of dry matter in grain in modern varieties, their N-use efficiency is also superior to that of old varieties. Physiological efficiency, a ratio of grain dry matter to total N accumulation by the plant, was greater for modern varieties than old varieties at all N levels (Tables 3.5b & 3.8), showing that modern varieties more efficiently convert accumulated N into harvestable yield.

The difference in the way the varieties partitioned dry matter was reflected by the partitioning of N between the straw and grain of the plant. Grain N accumulation and grain dry weight were correlated (r=.93 on residuals, r=.98 on means), as were straw N accumulation and straw dry weight (r=.81 on residuals, r=.95 on means). Modern varieties had more N in grain and less N in straw than old varieties (Tables 3.5a & 3.6). Modern varieties used more than half of their N in grain, while old varieties used less than half of their N in grain.

Semi-dwarf and tall varieties accumulated similar amounts of N (Tables 3.4 & 3.6), however, semi-dwarf varieties produced significantly more grain than tall varieties at a N rate of 135 kg ha^{-1} (Tables 3.5b & 3.8). Semi-dwarf varieties also had more N in grain and less in straw than did tall varieties (Tables 3.5b & 3.6), indicating that semi-dwarf varieties partitioned more of their N reserves in grain than did tall varieties. The harvest indices for semi-dwarf and tall varieties show that semi-dwarf varieties also partitioned more of their dry matter to grain than did tall varieties (Tables 3.5b & 3.8). Despite the greater portion of dry matter in grain in semi-dwarf varieties, their N-use efficiency (physiological efficiency) was not greater than that of the tall varieties, except at a N rate of 67 kg ha^{-1} (Tables 3.5b & 3.6).

Many researchers have suggested that modern semi-dwarf varieties require higher N rates than taller varieties (Brandon and Wells, 1986; De Datta *et al.*, 1968; Guindo *et al.*, 1994b; Hartley and Milthorpe, 1982; Roberts, *et al.*, 1993; Tran Van Dat *et al.*, 1978; Wells and Turner, 1984). If so, semi-dwarf varieties would either produce less grain than tall varieties at the same N rate, or produce more grain at higher rates. At the 135 kg ha^{-1} N rate, this was not observed in 1995 (Table 3.9). Data from numerous years of variety trials in Crowley, LA, do not indicate that semi-dwarf varieties respond to N rates above 135 kg ha^{-1} with higher grain yields (Table 3.8). While there were rarely significant differences, semi-dwarf varieties Lacassine, Lemont, Maybelle, and RT 7015 generally had a lower yield with the 168 kg N ha^{-1} treatment than the 135 kg ha^{-1} treatment. Cypress and Jodon generally had a higher yield (also semi-dwarf varieties). Tall varieties had a lower yield with

Table 3.9: Grain yield of modern long grain varieties tested at two N levels at Crowley, LA. Mean separations of grain yield at the two N levels for one variety in one year are done with a protected LSD (**=p≤.01; ns=not significant).

VT[1]	V[2]	N rate	Grain yield at 12% moisture					
			1994[3]	1993[4]	1992[5]	1991[6]	1990[7]	1985[8]
		kg ha^{-1}	kg ha^{-1}					
TE	KB	135	9520	8964				
		168	9170	9206				
			**	ns				
	KT	135				8501	8467	
		168				7548	8276	
						**	ns	
	NB	135						8486
		168						8766
								?
TV	AD	135	9137	9839	10213			
		168	8457	9572	9987			
			ns	ns	ns			
	JK	136	9455	10392	9573	9562		
		168	9114	10445	9730	9624		
			ns	ns	ns	ns		
	TB	135			9650	8731	8870	7141
		168			9092	8682	8699	6928
					**	ns	ns	?
DE	CP	135	9758	9673	9272	9654		
		168	9952	10213	9647	9458		
			ns	ns	ns	ns		
	LC	135		9534	9215	9988		
		168		9125	9095	9988		
				ns	ns	ns		
	LM	135	9372	8795	8984	9685	8900	9170
		168	9150	8495	8554	9263	8471	9865
			ns	ns	ns	ns	ns	?
DV	JD	135	9854	9996				
		168	10082	10014				
			ns	ns				
	MB	135	8341	10058	9631	8418	7736	
		168	8085	10465	8271	8317	7267	
			ns	ns	**	ns	ns	
	RT	135		9338	9798	9243		
		168		9052	9604	9735		
				ns	ns	ns		
Maximum C.V. (%)			1.4	5.4	7.8	5.2	5.1	5.9

[1]Variety types, TE=tall/early, TV=tall/very early, DE=semi-dwarf/early, DV=semi-dwarf/very early; [2]Varieties, KB=Kaybonnet, KT=Katy, NB=Newbonnet, AD=Adair, JK=Jackson, TB=Tebonnet, CP=Cypress, LC=Lacassine, LM=Lemont, JD=Jodon, MB=Maybelle, RT=RT 7015; [3]Bollich *et al.*, 1994b; [4]Bollich *et al.*, 1993b; [5]Bollich *et al.*, 1992b; [6]Bollich *et al.*, 1991a; [7]Bollich *et al.*, 1990a; [8]Brandon *et al.*, 1985a.

the higher N rate most years, with the notable exception of Jackson, which appeared to respond to the higher N rate. From these data under Louisiana conditions, it is difficult to conclude that semi-dwarf varieties respond to higher N rates, and it seems appropriate to conclude that 135 kg N ha^{-1} is an optimum rate for most of the modern long grain varieties in this experiment.

There are numerous reasons why researchers have suggested that semi-dwarf varieties require higher N rates than taller varieties. Some researchers do not provide methods or sufficient data to support their suggestion (Wells and Turner, 1984), making it difficult to evaluate their results. Many studies are based on a comparison of one or more semi-dwarf varieties with one or two tall varieties (Hartley and Milthorpe, 1982; Brandon and Wells, 1986; Guindo *et al.*, 1994b). As was noted above, there was great variability in N accumulation and plant growth among tall varieties, but little with semi-dwarf varieties. In basing conclusions on a comparison of one or two varieties of a type that has great variability, one risks selecting an individual variety that will give comparative results that are not consistent with the majority of varieties in their class. Furthermore, in making a comparison with a variety type that has great variability, perhaps it would be better not to generalize for all or even most varieties of that type, and probably best not to generalize about that variety type at all. It would certainly not be statistically appropriate to generalize conclusively if the varieties selected were not selected at random from all varieties of that type. One study reported lodging and the inability of their harvester to recover as much grain from lodged as unlodged plants (Hartley and Milthorpe, 1982). In other studies (Tran Van

Dat *et al.*, 1978; Roberts *et al.*, 1993), a combine was used for harvest, but no mention was made about lodging or the combine's ability to harvest all above-ground plant material. Still other studies give no indication of harvest method (De Datta *et al.*, 1968; Brandon and Wells, 1986). If all above-ground plant material is not harvested, then an accurate measurement of N accumulation or dry matter cannot be made. Since tall varieties frequently lodge while semi-dwarf varieties do not, N accumulation and dry matter yield could be underestimated for tall varieties. As a consequence of comparing inaccurate measurements of tall varieties with accurate measurements of semi-dwarf varieties, one could either erroneously conclude that the variety types differ, or one could correctly conclude that the variety types differ, but on the basis of erroneous data. In either case, the conclusion would not be reliable.

A final consideration that might explain differences reported between semi-dwarf and tall varieties is climate. Most of the studies were conducted in areas with a cooler climate than Louisiana. Variety response to N rate might be affected by temperature, such that at lower temperatures, some semi-dwarf varieties might respond to higher N rates, while at higher temperatures, the same varieties might not respond to higher N rates. Cypress, a semi-dwarf variety which frequently yielded more grain with the 168 kg N ha^{-1} treatment than the 135 kg ha^{-1} treatment (Bollich *et al.*, 1992b, 1993b, 1994b), was shown to accumulate more N in a cooler year than a warmer year, but with no accompanying change in grain yield (Chapter 1).

Comparisons made thus far have involved old varieties and modern long-grain varieties. However, all of the old varieties were medium-grain. Therefore, three

modern medium-grain varieties also were compared to the modern long-grain and old varieties already used in this experiment. The modern medium-grain varieties all matured early, and represented both semi-dwarf and tall plants. The experimental data were reanalyzed with the addition of data for the modern medium-grain varieties, and varieties were placed in three variety-type groups: modern medium-grain, modern long-grain (combined), and old.

Unlike comparisons made without the modern medium-grain varieties (Table 3.4), these three groups differed in total N accumulation, as well as all other factors, except total N accumulation early in the season (Table 3.10). Total N accumulation by medium-grain varieties late in the season was greater than both long-grain and old varieties at 135 kg N ha^{-1} (Table 3.11), whereas long-grain and old varieties did not differ (Table 3.4). Likewise, grain yield of medium-grain varieties at 135 kg N ha^{-1} was greater than both long-grain and old varieties (Table 3.12). The harvest index and the physiological efficiency of medium-grain and long-grain varieties did not differ (Table 3.12). This suggests that medium-grain varieties, similar to long-grain varieties, have the same desirable ability to partition more N to grain than straw. Since the medium-grain varieties were able to accumulate more N than the other varieties from the same soil, they might have a superior ability to absorb N from soil. This difference developed later in the season, as there was no difference in total N accumulation early in the season (Table 3.10). Furthermore, the difference between medium-grain varieties and the other varieties becomes more pronounced at higher N levels and later in the season (Table 3.11). The difference in ability to absorb N from soil could be due

Table 3.10: Analysis of variance of the main effects and interactions in the field experiment. Variety-types considered were old, modern long-grain, and modern medium-grain varieties.

	Field, early		Field, late								Field	
			Dry matter			N Accumulation						
Effects	Dry Matter Total	N Accu. Total	Straw	Grain	Total	Straw	Grain	Total	HI[1]	PE[2]	HD[3]	HT[4]
Level	**	**	**	**	**	**	**	**	**	**	**	**
Type	*	ns	**	**	**	**	**	**	**	**	**	**
Variety(Type)	**	ns	**	**	**	*	**	*	**	**	**	**
Level x Type	ns	ns	ns	**	ns	ns	**	ns	**	*	**	*
Level x Variety(Type)	ns	ns	ns	ns	ns	ns	*	ns	ns	ns	**	**
C.V. (%)	19.5	20.8	13.5	11.1	10.7	18.7	12.1	12.5	6.9	8.8	1.2	5.5

Significance of F-values: *p≤.05; **p≤.01; ns=not significant.
[1]Harvest index (grain yield / total yield).
[2]Physiological efficiency (grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}).
[3]Days to 50% heading.
[4]Plant height at maturity.

Table 3.11: Nitrogen accumulation and related means of all varieties of a variety-type. Medium-grain varieties are the control to which long-grain and old varieties are compared.

Varieties Compared	Field, early		Field, late					Field			
	N Accumulation		N Accumulation					Days to 50%		Plant Height at	
	Total		Straw		Grain		Total	Heading		Maturity	
	kg ha^{-1}									cm	
at 0 kg N ha^{-1}:											
medium-grain	15.3		25.8		39.2		64.7	87.8		72.4	
long-grain	14.5	ns	23.6	ns	38.3	ns	61.8	ns	83.9	**	70.1	ns
old	16.9	ns	31.4	ns	32.9	ns	64.8	ns	95.0	**	98.0	**
at 67 kg N ha^{-1}:											
medium-grain	61.5		47.2		71.8		119.1		85.1		83.5	
long-grain	60.9	ns	45.9	ns	67.6	ns	113.5	ns	82.2	**	81.6	ns
old	61.2	ns	54.8	ns	52.6	**	108.0	ns	91.2	**	113.3	**
at 135 kg N ha^{-1}:												
medium-grain	108.2		78.9		92.3		171.2		87.3		94.6	
long-grain	99.1	*	69.6	**	86.4	ns	156.0	**	85.4	**	92.4	ns
old	95.6	**	80.5	ns	65.7	**	146.3	**	91.7	**	127.0	**

Means of long-grain and old varieties are compared to medium-grain varieties at the same N level by Dunnett's test, *p≤.05, **p≤.01, ns=not significant.

Table 3.12: Dry matter accumulation and related means of all varieties of a variety-type. Medium-grain varieties are the control to which long-grain and old varieties are compared.

Varieties Compared	Field, early		Field, late									
	Dry Matter		Dry matter						Harvest		Physiological	
	Total		Straw		Grain		Total		Index[1]		Efficiency[2]	
	Mg ha^{-1}								ratio			
at 0 kg N ha^{-1}:												
medium-grain	.98		4.38		4.16		8.53		.49		63.8	
long-grain	.87	ns	3.77	ns	3.74	ns	7.50	ns	.50	ns	60.5	ns
old	.89	ns	5.97	**	3.06	**	9.03	ns	.33	**	46.2	**
at 67 kg N ha^{-1}:												
medium-grain	2.93		8.58		7.22		15.80		.46		61.1	
long-grain	2.74	ns	7.46	**	6.54	**	13.99	**	.47	ns	57.8	ns
old	2.87	ns	10.57	**	5.39	**	15.96	ns	.34	**	50.3	**
at 135 kg N ha^{-1}:												
medium-grain	3.55		11.17		8.61		19.78		.44		50.6	
long-grain	3.12	*	10.29	*	7.95	*	18.23	**	.44	ns	51.0	ns
old	3.01	*	13.08	ns	6.19	**	19.26	ns	.32	**	42.4	**

Means of long-grain and old varieties are compared to medium-grain varieties at the same N level by Dunnett's test, *p≤.05, **p≤.01, ns=not significant.

[1]Grain yield / total yield.

[2]Grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}.

to differences in the root system, the rhizosphere oxidizing ability of the roots, or because of more efficient transport of N across root membranes.

Although only three medium-grain varieties were used, contrasts of varieties within each variety-type indicate that the three medium-grain varieties did not differ from one another in either total N accumulation or grain yield (Table 3.13). Differences existed only in plant height and straw dry matter. The data show that there are differences between modern medium-grain varieties and long-grain varieties in total N accumulation, grain yield, and other factors late in the season, and suggest that these differences might be more dramatic than differences between semi-dwarf and tall long-grain varieties.

Conclusions

Total N accumulation in old and modern long-grain varieties of rice was similar from a preflood N application of 0, 67, or 135 kg ha^{-1}. However, long-grain varieties produced more grain than old varieties at all N levels, and among the long-grain varieties, semi-dwarf varieties produced more grain than tall varieties at 135 kg N ha^{-1}.

Early maturing and very early maturing varieties did not differ. Long-grain varieties, and semi-dwarf varieties in particular, produced higher grain yield without higher N accumulation by partitioning more of their N to grain than straw. This suggests that long-grain varieties are more efficient at converting N into grain, and therefore should be considered for maximum yields, particularly at low N rates.

Grain yield of semi-dwarf long-grain varieties was higher than tall varieties at 135 kg N ha^{-1}. Therefore, the hypothesis that semi-dwarf varieties require higher N

Table 3.13: Significance of comparisons of the means of varieties of a variety-type. Variety-types considered were old, modern long-grain, and modern medium-grain.

	Field, early		Field, late								Field	
			Dry Matter			N Accumulation						
Variety type	Dry Matter Total	N Accu. Total	Straw	Grain	Total	Straw	Grain	Total	HI[1]	PE[2]	HD[3]	HT[4]
medium-grain	ns	ns	**	ns	ns	*	ns	ns	**	**	ns	**
long-grain	**	ns	**	**	**	*	**	ns	**	**	**	**
old	ns	ns	ns	**	**	ns	**	ns	**	**	**	**

Significance of F-values: *p≤.05; **p≤.01; ns=not significant.
[1]Harvest index (grain yield / total yield).
[2]Physiological efficiency (grain yield in kg ha^{-1} / total N accumulation in kg ha^{-1}).
[3]Days to 50% heading.
[4]Plant height at maturity.

rates than taller varieties was not supported, although semi-dwarf varieties might respond to higher N rates than certain tall varieties.

Modern medium-grain varieties accumulated more N than old and long-grain varieties, and at 67 and 135 kg N ha^{-1}, produced more grain than the others. Medium-grain varieties had a higher harvest index and physiological efficiency than old varieties, but did not differ from long-grain varieties. This suggests that both long-grain and medium-grain varieties have the desirable ability to partition more N to grain than straw, while possibly having a superior ability to absorb N from soil.

CHAPTER 4

COMPARISON OF AMMONIUM SULFATE AND UREA AS NITROGEN SOURCES FOR RICE

Introduction

Nitrogen is considered the major yield-limiting factor in flooded rice production (De Datta, 1981; Fageria, *et al.*, 1991; von Hexküll and Beaton, 1992). Although rice will use both ammonium and nitrate forms of N, only ammonium is stable under flooded conditions (Bagent *et al.*, 1987). Urea ($CO(NH_2)_2$), AS ($(NH_4)_2SO_4$), or other non-nitrate N fertilizers are generally used with flooded rice, since NO_3^--N is subject to denitrification losses in submerged soils (Brandon and Wells, 1986). Wells and Turner (1984) suggested that NO_3^--N could be almost as effective as NH_4^+-N when applied in small amounts (less than 30 kg ha^{-1}) at PI. At PI, a root mat has commonly formed at the soil surface, so that plants are able to absorb the applied N. However, Wilson *et al.* (1994) compared granular urea to a urea-ammonium nitrate solution applied in a three-way split, and found the highest fertilizer N uptake and grain yield when granular urea was the N source.

Several studies have compared the effectiveness of AS and urea. An early study by Patrick *et al.* (1967) reported little difference in grain yield at maturity between AS and urea. A later study (Reddy and Patrick, 1978), with ^{15}N in small microplots, found no differences in straw or grain yield at maturity between the two N sources. A greenhouse study comparing urea, anhydrous ammonia, and AS (Sarkar *et al.*, 1978) found the highest N uptake and fertilizer use efficiency from AS. Grain

yields were highest with AS at 60 kg N ha^{-1}, but with urea at 120 kg N ha^{-1}. In a greenhouse study, Phongpan *et al.* (1988) found no differences in grain and straw yields between urea and AS applied to an acid sulfate soil at a low N rate (800 mg N per 5 kg soil), but at higher N rates (1600 and 2400 mg N per 5 kg soil), urea consistently produced higher yields than AS. Fertilizer N recovery was greater when AS rather than urea was applied at transplanting, but was less when urea was applied at PI. None of these studies considered the possible contribution of S to plant growth.

Blair (1987) reported a response to S applied with N in flooded soils, and indicated that S deficiency, much the same as N deficiency, reduces grain yield by reducing tillering, number of panicles, grains per panicle, and grain weight. Sulfur deficiency is being reported with increasing frequency because of an increase in use of high-purity, low-S fertilizers, and use of high-yielding cultivars that have a greater S requirement, as well as a decrease in use of farmyard manure and S-containing pesticides (Blair, 1987; Blair *et al.*, 1978; Freney *et al.*, 1982; Wang, 1978).

Several studies have examined the effectiveness of S sources. Blair *et al.* (1979) and Samosir *et al.* (1983) reported no difference in rice yields when gypsum ($CaSO_4 \cdot 2H_2O$), elemental S, or AS were broadcast as S sources at transplanting. Chien *et al.* (1987) reported higher grain yields and S uptake with gypsum than with elemental S, both broadcast Since S is absorbed by rice as sulfate (Blair *et al.*, 1978; Freney *et al.*, 1982; Wang, 1978), it is important that elemental S not be incorporated into the reduced soil layer, where oxidation to sulfate will not take place (Blair, 1987; Blair *et al.*, 1978; Wang, 1978). Chien *et al.* (1987) reported that rice yield and S

uptake were the same when elemental S was either broadcast or surface-incorporated, but that yield and uptake were less when elemental S was deep-placed. Higher yields have also been obtained with surface-incorporated elemental S and from surface-incorporated gypsum when compared with deep-placed elemental S (Chien *et al.*, 1988). Similarly, Samosir *et al.* (1993) found that broadcast elemental S, broadcast potassium sulfate, and deep-placed potassium sulfate produced yields that were greater than those obtained with deep-placed elemental S (Samosir *et al.*, 1993).

The objectives of this study were (1) to compare the effectiveness of urea, urea plus elemental S, and AS as sources of N in flooded rice culture, (2) to compare fertilizer recovery from application of each N source at PF and PI, and (3) to determine if there is a response to S by rice grown on a soil with a less than optimum level of available S.

Materials and Methods

Field experiments were conducted in 1993 and 1994 at the Rice Research Station in Crowley, LA, on a Crowley silt loam (fine, montmorillonitic, thermic Typic Albaqualf). Soil chemical and physical properties are summarized in Table 4.1. 'Cypress' rice was drill-seeded in 17.8 cm rows at 112 kg ha^{-1} in 2.1 x 7.6 m field plots. Prior to permanent flooding at the four-leaf stage of plant growth, microplots were established within each field plot. Microplots consisted of 61 cm diameter polyvinyl chloride rings, 30 cm in height, driven 12-15 cm into the soil to the depth of a clay pan. Urea, urea plus S, or AS was broadcast on the soil surface at 101 kg N ha^{-1}. Field plots received unlabeled N, while microplots received either unlabeled N or ^{15}N-labeled N

Table 4.1: Initial soil chemical and physical properties of a Crowley silt loam soil at the Rice Research Station, Crowley, LA, used in a fertilizer source experiment.

Soil property	1993	1994
pH	6.5	5.8
Organic matter (%)	1.41	1.38
extractable P (mg kg^{-1})[1]	65	26
exchangeable Na (mg kg^{-1})[2]	76	77
exchangeable K (mg kg^{-1})[2]	68	75
exchangeable Ca (mg kg^{-1})[2]	1496	1171
exchangeable Mg (mg kg^{-1})[2]	200	205
extractable Zn (mg kg^{-1})[3]	0.94	2.97
extractable S (mg kg^{-1})[4]	7.25	10.75
extractable As (mg kg^{-1})[5]	2.21	1.23
extractable Fe (mg kg^{-1})[3]	57.61	103.2

[1]Bray-2
[2]Ammonium acetate at pH 7.0
[3]Diethylene triamine pentaacetic acid
[4]Acetic acid and ammonium acetate
[5]Hydrocholoric acid

(2.23 atom% AS-^{15}N and 5.02 atom% urea-^{15}N in 1993, 2.85 atom% AS-^{15}N and 2.22 atom% urea-^{15}N in 1994). Elemental S was broadcast on the soil surface at 173 kg S ha^{-1}. The field was flooded and maintained throughout the experiment. At PI, urea or AS was broadcast into the floodwater at 50 kg N ha^{-1}. Field plots received unlabeled N, while those microplots which received unlabeled N PF received labeled N, and those microplots which received labeled N PF received unlabeled N. Treatments are summarized in Table 4.2. The experimental design was a randomized complete block, with three replications in 1993 and four replications in 1994.

Microplots were harvested by hand at 50% heading. Three 30 x 30 cm areas from each field plot were harvested at maturity. In both cases, all above-ground plant material was harvested.

Plants were oven-dried at 60°C to a constant weight. Samples collected at maturity were separated into grain and straw. Plant material was ground in a Wiley mill to pass through a 1 mm mesh sieve. Subsamples of dried plant material were digested in concentrated sulfuric acid. The semi-micro Kjeldahl procedure was followed for total N determination (Bremner and Mulvaney, 1982). Ethanol (95%) was distilled between samples to prevent ^{15}N cross-contamination during distillation. Distillates were acidified with 2 mL of .08 *N* hydrochloric acid and evaporated to dryness for ^{15}N analysis (Hauck, 1982). Nitrogen 15 atom% was determined on a Du Pont 21-621 mass spectrometer.

Data were analyzed with the SAS System (SAS Institute, 1994). Analysis of variance with the GLM procedure was used to determine if significant differences and

Table 4.2: Summary of treatments.

	Nitrogen Application			
	Preflood		Panicle Initiation	
Fertilizer source	Unlabeled	Labeled	Unlabeled	Labeled
	kg N ha^{-1}			
ammonium sulfate	101	0	0	50
ammonium sulfate	0	101	50	0
urea	101	0	0	50
urea	0	101	50	0
urea + sulfur†	101	0	0	50
urea + sulfur†	0	101	50	0

†173 kg elemental S ha^{-1} preflood

interactions existed among treatments. Tukey's test (α=.05) was used to compare means. Assumptions for the validity of the analyses were checked.

Results and Discussion

Under the conditions of this study, AS, urea, and urea plus S were equally effective for rice production. Similar results were obtained by Patrick *et al.* (1967), Reddy and Patrick (1978), and Sarkar *et al.* (1978). Plant dry matter at 50% heading did not differ among years, N fertilizers, and times of labeled N application (Table 4.3). One would not expect differences between times of labeled N application, because the same total N was applied in each case, and labeled and unlabeled N are functionally the same. Although dry matter accumulation is often affected by climate, no differences were detected during the two years of this study. There was a year x time interaction for dry matter (Table 4.3), but when means were compared with Tukey's test, no differences were found (Table 4.4). Grain and total dry matter at maturity were not affected by N fertilizer or time of N application (Table 4.5). Grain yield is particularly important to the grower, so the lack of difference among N fertilizers suggests that either AS or urea can be used with equal agronomic effectiveness. While there was no significant difference in straw dry matter at maturity between AS and urea, and urea and urea plus S, there was a difference between AS and urea plus S (Table 4.6). This suggests that urea-N might be more effective than NH_4^+-N in straw production, although this would not be important to the grower. Furthermore, grain and straw yields were generally greater when urea plus S were applied than when other fertilizers

Table 4.3: Results from the analysis of variance of the effects of year, fertilizer source, and time of application of labeled N on plant growth and nitrogen accumulation at 50% heading.

Effects	Plant Dry Matter	Total N Accumulation	Labeled Fertilizer N Accumulation			Labeled Fertilizer N Recovery
	93-94	93-94	93-94	93	94	93-94
Year	ns	**	ns			ns
Fertilizer	ns	ns	ns	*	ns	ns
Time of labeled N	ns	ns	**	**	**	**
Year x Fertilizer	ns	ns	ns			ns
Year x Time	*	ns	ns			ns
Fertilizer x Time	ns	ns	**	**	ns	*
Year x Fertilizer x Time	ns	ns	*			ns
C.V. (%)	5.4	9.5	15.7	10.3	18.5	19.5

Significance of F-values: *=p≤.05; **=p≤.01; ns=not significant.

Table 4.4: Treatment means for plant growth and nitrogen accumulation at 50% heading.

Treatments	Plant Dry Matter	Total N Accumulation	Labeled Fertilizer N Accumulation		Labeled Fertilizer N Recovery
	Mg ha^{-1}	———	kg ha^{-1}	———	%
	93-94	93-94	93	94	93-94
Year					
1993	11.00 a	99.8 a			40.3 a
1994	11.29 a	141.2 b			40.0 a
Fertilizer x Time of labeled N					
Ammonium sulfate, PF	11.42 a	112.4 a	26.2 b	34.9 ab	30.3 c
Ammonium sulfate, PI	11.09 a	120.9 a	26.8 b	23.6 bc	50.0 a
Urea, PF	11.21 a	126.4 a	37.3 a	36.8 a	36.8 bc
Urea, PI	10.86 a	111.9 a	18.4 b	23.6 bc	41.7 abc
Urea plus S, PF	11.20 a	127.0 a	41.3 a	34.0 ab	37.4 abc
Urea plus S, PI	11.12 a	124.2 a	24.2 b	21.0 c	44.9 ab

Means within the same column (for year or fertilizer x time interaction) followed by the same letter are not significantly different at α=.05 by Tukey's test.

Table 4.5: Results from the analysis of variance of the effects of year, fertilizer source, and time of application of labeled N on plant growth and nitrogen accumulation at maturity. There were no significant interactions (α=.05).

	Dry Matter			Total N Accumulation		
Effects	Straw	Grain	Straw plus Grain	Straw	Grain	Straw plus Grain
Year	*	**	**	ns	ns	ns
Fertilizer	*	ns	ns	ns	ns	ns
Time of labeled N	ns	ns	ns	ns	ns	ns
C.V. (%)	9.7	8.7	8.8	16.2	10.5	11.7

Significance of F-values: *=p≤.05; **=p≤.01; ns=not significant.

Table 4.6: Treatment means for plant growth and nitrogen accumulation at maturity.

	Dry Matter			Total N		
Treatment	Straw	Grain	Straw plus Grain	Straw	Grain	Straw plus Grain
	Mg ha^{-1}			kg ha^{-1}		
Year						
1993	10.50 a	8.99 a	19.49 a	44.1 a	94.8 a	138.9 a
1994	9.83 b	8.19 b	18.01 b	45.6 a	93.0 a	138.5 a
Fertilizer						
Ammonium sulfate	9.63 a	8.51 a	18.14 a	41.1 a	92.2 a	133.3 a
Urea	10.21 ab	8.47 a	18.67 a	45.9 a	92.6 a	138.4 a
Urea plus S	10.65 b	8.79 a	19.45 a	47.5 a	96.9 a	144.4 a
Time of labeled N						
PF	10.41 a	8.66 a	19.07 a	46.0 a	96.4 a	141.4 a
PI	9.92 a	8.52 a	18.44 a	43.6 a	92.4 a	136.0 a

Means within the same column (for year, fertilizer, or time of labeled N) followed by the same letter are not significantly different at α=.05 by Tukey's test.

were used (Table 4.6). Both grain and straw dry matter yields were higher in 1993 than in 1994, however, there were no interactions with year (Table 4.6).

Total N accumulation at 50% heading did not differ with N fertilizers or time of labeled N application (Table 4.3). There was a difference between years, with considerably more N accumulation in 1994 (Table 4.4). However, there were no interactions with year. Total N accumulation at maturity did not differ among years, N fertilizers, or times of labeled N application (Table 4.5). The plots treated with AS had lower N accumulations than those treated with urea, and those treated with urea had lower N accumulations than those treated with urea plus S, both at 50% heading (Table 4.4) and at maturity (Table 4.6). Those differences, which were the result of the natural variability of the plant population, carried over to dry matter accumulations. However, dry matter accumulation is not absolutely correlated with total N accumulation, as evidenced by the absence of the trend for plant dry matter at 50% heading (Table 4.4).

Since 101 kg N ha^{-1} was applied PF while only 50 kg N ha^{-1} was applied at PI, a difference in labeled fertilizer N accumulation between PF and PI applications was expected. Because there was a year x fertilizer x time interaction for labeled fertilizer N measured at 50% heading, the results were analyzed separately for each year. The expected difference between PF and PI was seen in 1994, with 35.2 kg ^{15}N ha^{-1} accumulation from PF application and 22.7 kg ^{15}N ha^{-1} from PI application. However, there was a fertilizer x time interaction in 1993, such that the expected difference between PF and PI application was seen only with urea and urea plus S (Table 4.4).

Accumulation of labeled fertilizer N from AS did not differ between PF and PI application in 1993. This indicates that fertilizer recovery from PF application was about half of that from PI application of AS (25.9% *vs.* 53.6%), whereas it was only slightly less from PF than from PI application of the other N fertilizers. The unusually low labeled N accumulation from the PF AS treatment in 1993 might be due to pool substitution, which occurs when fertilizer N exchanges with native soil N (Hart *et al.*, 1986; Jenkinson *et al.*, 1985). Although the amount of plant available N does not change, the source of the N does. Since this substitution occurs randomly, the effect among years, N fertilizers, and times of N application can differ, making it difficult to describe and explain trends in labeled N accumulation. Ignoring the PF AS data in 1993, the results show that N fertilizer had no effect on labeled fertilizer N accumulation, whereas time and amount of application did have an effect.

Labeled fertilizer N recovery did not differ among years or N fertilizers, but did differ among times of labeled N application (Table 4.3). Labeled fertilizer N recovery did not differ between PF and PI N applications for urea or urea plus S, but did differ for AS (Table 4.4). Means were generally higher for PI than PF (Table 4.4), and the variability was high (Table 4.3). Pool substitution could explain this variability. For AS, PI N application resulted in a 50% recovery, but this value was influenced by the unusual labeled fertilizer N accumulation in 1993.

There was no response to S. Where differences did occur (labeled fertilizer N applied at PF in 1993), the difference was not between S-containing and S-free fertilizers, but between AS and urea fertilizers (Table 4.4). The absence of a response

to S cannot be attributed to optimum S fertility, as this was not the case (Table 4.1). Louisiana soil test S of Coastal Prairie silt loams (such as the Crowley silt loam) is considered low when below 10 mg kg^{-1} of extractable S and medium when between 10 and 18 mg kg^{-1} (E.R. Funderburg, 1995, personal communication). The Louisiana analysis follows the method described by Tabatabai (1982) using ammonium acetate and acetic acid as extractants. Tiwari *et al.* (1983) reported a critical S level of 11 mg kg^{-1} using the same method on numerous Indian rice soils. Islam and Ponnamperuma (1982) reported a critical S level of 30 mg kg^{-1} using only ammonium acetate as an extractant on Philippine rice soils. Unpublished data from a 1994 statewide survey in Louisiana showed that Crowley silt loam soils in rice production in Acadia Parish (location of the present study) averaged 9.8 mg S kg^{-1} (P.F. Bell, 1995). This indicates that the fields used in the present study were representative of fields used for rice production, and because of their low S levels, suggests that a response to S should have occurred. However, Crowley silt loam soils have a subsurface clay pan which can limit leaching losses of SO_4^--S (Clark *et al.*, 1962). In addition, mineralization of organ S could be a source of available S. Finally, rice has an extensive root system that is able to mine a large area for nutrients (Beyrouty *et al.*, 1988; Beyrouty *et al.*, 1996; Slaton *et al.*, 1990). These conditions could offset the limited S availability in the soil.

Conclusions

This study showed that AS, urea, and urea plus S are equally effective in flooded rice production on a Crowley silt loam soil. Grain yields did not differ as a result of N fertilizer. It is likely that there would be greater N use efficiency of N

applied at PI than at PF. No response to S was seen, despite the low soil levels of available S. Unless S deficiency symptoms are apparent, AS and urea plus S would not be cost effective alternatives to urea. Therefore, it is unlikely that S-containing N sources would be of benefit on a Crowley silt loam soil.

CHAPTER 5

MICROPLOT SIZE AND RETAINER EFFECTS ON RICE GROWTH AND ^{15}N ACCUMULATION

Introduction

Numerous studies have addressed the availability, transformations, accumulation, and fate of N in flooded rice production (De Datta *et al.*, 1989; Patrick and Reddy, 1976; Simpson *et al.*, 1984; Westcott *et al.*, 1986). Many of these studies used N sources containing the stable ^{15}N isotope. The high cost of ^{15}N field studies, however, has confined their use to microplot experiments. The small size of microplots can result in a loss of accuracy in field measurements. In field situations, there is concern that ^{15}N will migrate with floodwater or soil water beyond the area of the microplot. In order to control ^{15}N migration, microplots usually are enclosed with impermeable retainers. This requires otherwise unnecessary labor to install the retainers and maintain floodwater levels in them. It also introduces undefined effects on the microclimate of the microplot and on plant growth.

Studies on border effects in experimental rice plots indicate that a border of two 20 cm rows is sufficient to offset the effects of unplanted borders (Gomez and DeDatta, 1971) or different cultivars (Gomez, 1972). Vernetti *et al.* (1982) investigated the border effect in a N fertility study, and found that the needed border ranged from 25 to 75 cm depending on cultivar and N rate. The results of these studies suggest that migration of fertilizers, N included, could be as little as 25 cm. With a minimal border effect, the use of retainers could be eliminated.

In an Australian study of microplot size and retainer effects, Trevitt *et al.* (1988) found that opaque retainers had a shading effect on the floodwater of 54 x 54 cm microplots. When rice plants were 8 to 10 weeks old, shading decreased light and heat penetration in microplots, resulting in lower temperature and pH, and a reduced rate of urea hydrolysis. Without rice plants, microplots with transparent retainers (54 x 54 cm microplots) were similar in temperature, pH, and ammoniacal N concentration to a field plot, while microplots with galvanized iron retainers (both 54 x 54 cm and 33 cm diameter microplots) had lower temperature and pH, and higher ammoniacal N concentration than a field plot.

Several studies in the rice-growing areas of the mid-southern United States have utilized ^{15}N and microplots (Bollich *et al.*, 1994a; Guindo *et al.*, 1994a; Reddy and Patrick, 1978). However, the accuracy of microplots in simulating open field conditions has not been addressed. Therefore, the objectives of this study were to (1) determine the effects of microplot size on plant growth, N accumulation, floodwater and soil temperature, and floodwater and soil pH, (2) compare plant growth, N accumulation, floodwater and soil temperature, and floodwater and soil pH of microplots with and without retainers, and (3) determine the size of microplots with and without retainers that most closely simulate a field plot.

Materials and Methods

Field experiments were conducted in 1993 and 1994 at the Rice Research Station in Crowley, LA, on a Crowley silt loam (fine, montmorillonitic, thermic Typic Albaqualf). Soil chemical and physical properties are summarized in Table 1.1.

'Cypress' rice was drill-seeded in 17.8 cm rows at 112 kg ha^{-1}. Microplots were established at the three-leaf stage of plant development. Circular microplots (15, 35, and 60 cm diameter) with polyvinyl chloride retainers, and square microplots (75 cm length) with and without stainless steel retainers were completely randomized and replicated three times. In 1994, twelve 75 cm microplots were established, 6 for bulk harvesting, and 6 for harvest by row (two middle rows inside the retainer, two edge rows inside the retainer, and two rows immediately outside the retainer). There were no bulk-harvested 75 cm microplots in 1993. Retainers were inserted into the soil to a depth of 10 cm. Rectangular field plots 2.1 x 7.6 m were established adjacent to the microplots, and used as controls. Treatments are summarized in Table 5.1. Nitrogen 15-labeled urea (2.60 atom% in 1993, 2.22 atom% in 1994) was broadcast on all microplots at 151 kg N ha^{-1} at the three-leaf stage of plant development. Where retainers were not used, a retainer was placed on the soil surface to aid in accurate fertilizer placement, then removed. Unlabeled urea was broadcast on the field plots at 151 kg N ha^{-1}, and in 1994, to the areas around the microplots. The field was flooded, and the flood was maintained throughout the experiment.

In 1993, floodwater and soil temperature, and floodwater and soil pH were measured at approximately 0800, 1200, and 1600 h for three consecutive days beginning at 44 days after sowing (DAS) and at 70 DAS. Measurements were made with a portable pH meter (Orion Research, Inc., Boston, MA) at the center of each microplot just above the soil surface for water measurements and at a depth of

Table 5.1. Summary of microplot treatments.

microplot size (abbreviation)	retainer[1]	plants harvested	years planted
15 cm diameter (15R)	PVC	all	1993, 1994
35 cm diameter (35R)	PVC	all	1993, 1994
60 cm diameter (60R)	PVC	all	1993, 1994
75 x 75 cm (75R)	SS	all	1994
75 x 75 cm (RM)	SS	2 middle rows	1993, 1994
75 x 75 cm (RE)	SS	2 edge rows	1993, 1994
75 x 75 cm (RO)	SS	2 outside rows	1993, 1994
75 x 75 cm (75O)	none	all	1994
75 x 75 cm (OM)	none	2 middle rows	1993, 1994
75 x 75 cm (OE)	none	2 edge rows	1993, 1994
75 x 75 cm (OO)	none	2 outside rows	1993, 1994
2.1 x 7.6 m field	none	3 m of row	1993, 1994

[1] PVC=polyvinyl chloride, SS=stainless steel

approximately 5 cm for soil measurements. Frequent afternoon rains prevented collection of accurate data in 1994.

All plots were hand-harvested at 90% heading in both years. Average maximum extended heights of 15 individual plants per microplot were measured. In bulk microplots, all above-ground plant material was harvested. In microplots harvested by row, above-ground plant material was harvested only from the center 45 cm of each row. In field plots, a 3 m row of above-ground plant material was harvested from the interior of the plot. Soil samples were collected with a 1.8 cm diameter hand probe to a depth of 15 cm. Two cores were collected from each plot in 1993, and four cores in 1994.

Root samples were also collected with the hand probe to a depth of 15 cm. Four cores were collected from each plot and separated into 0 to 7.5 cm and 7.5 to 15 cm depth increments. Roots were separated from soil with a root washing system. Root length was determined with an edge discrimination technique developed by Pan and Bolton (1991), and root length densities were calculated for each sample.

Plant material was oven-dried to a constant weight at 60°C while soil samples were air dried. Samples were ground in a Wiley mill to pass a 1 mm mesh sieve. Subsamples of dried plant material and soil were digested in concentrated sulfuric acid. The semi-micro Kjeldahl procedure was followed for total N determination (Bremner and Mulvaney, 1982). Ethanol (95%) was distilled between samples to prevent ^{15}N cross-contamination during distillation. Distillates were acidified with 2 mL of .08*N*

hydrochloric acid and evaporated to dryness for ^{15}N analysis (Hauck, 1982). Nitrogen 15 atom% was determined on a Du Pont 21-621 mass spectrometer.

Data were analyzed with the SAS System (SAS Institute, 1994). Analysis of variance with the GLM procedure was used to determine if significant differences existed among the treatments. Dunnett's Test (α=.05) and Duncan's Multiple Range Test (α=.05) were used for mean separations. Assumptions for the validity of the analyses were checked.

Results and Discussion

Experiments conducted with large plots attempt to simulate open field conditions. Microplots are also used to simulate the growing environment of the open field. In this experiment, each microplot treatment was compared with a 2.1 x 7.6 m field plot.

Effect of Microplot Size

Plant height generally decreased as microplot size decreased (Fig. 5.1). In 1993, there was a significant difference in plant height between each treatment and the field plot. In 1994, only the plants in the 15 cm microplot were significantly shorter than those in the field plot.

Plant dry matter was not affected by microplot size (Fig. 5.1). Only the 35 cm microplot in 1993 was significantly different from the field plot, although the means for dry matter in most microplots were higher than that of the field plot. This suggests that shorter plants have as much or more herbage than taller plants. Therefore, plant height cannot be used as an indication of dry matter accumulation.

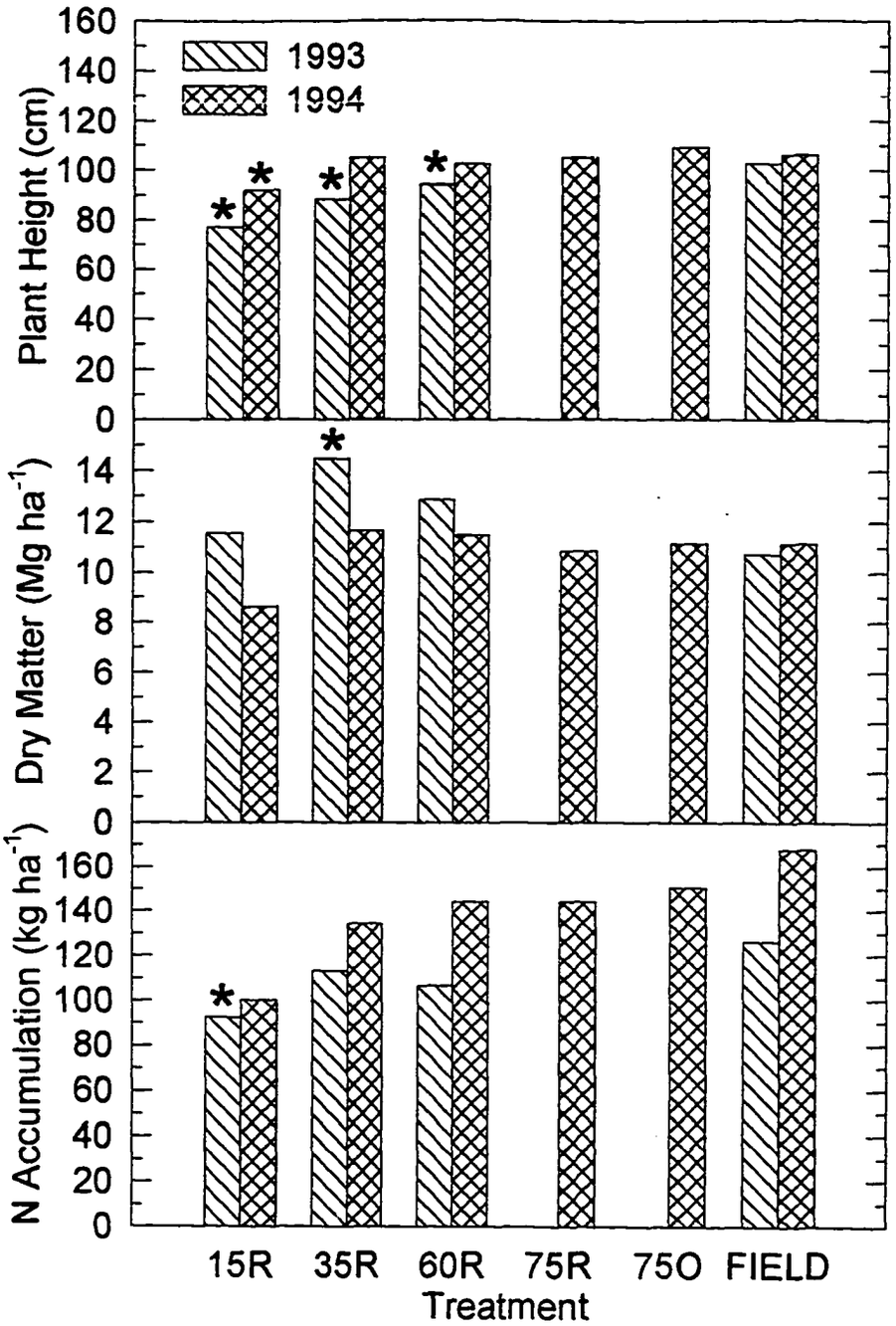

Fig. 5.1. Effect of microplot size on rice plant height, dry matter yield, and total N accumulation. Plot treatments are 15, 35, and 60 cm diameter microplots with retainers, 75 cm square microplot with a retainer, 75 cm square microplot without a retainer, and 2.1 x 7.6 m field plot. Means that differ from the field plot by Dunnett's test (α=.05) within the same year are marked (*).

While plants in many of the smaller microplots were shorter than those in the field plot, root length density was similar (Fig. 5.2).Values for root length density are similar to those reported in other studies (Beyrouty *et al.*, 1988; Slaton *et al.*, 1990). However, statistical differences in root length density are hard to detect because of the large variability among field samples. Coefficients of variation for the root samples ranged from 16.1 to 23.2%. Both root length density and plant height appeared to decrease with decreasing microplot size when retainers were used. This was more apparent at the 7.5-15 cm depth for root length density. However, in most microplots the means of root length density were about the same as or greater than those of the field plot, while the corresponding heights were lower than the field plot (Fig. 5.2). Since roots are the interface through which plants acquire nutrients, the plants' ability to take up N did not differ from the field plot regardless of microplot size.

Because root length density was similar among the treatments, poor root growth did not limit N uptake. With the exception of plants in the 15 cm microplot in 1993, total N accumulation did not differ among the treatments (Fig. 5.1). Total N accumulation was similar to values reported in other studies (Bollich *et al.*, 1994a; Guindo *et al.*, 1994a; Westcott *et al.*, 1986). As was the case with root length density, significant differences might not have been detected because of high CV (19.0% in 1993, 17.1% in 1994). However, total N accumulation decreased as microplot size decreased, and in all cases, was less than that in the field plot. Since root length densities were not lower than those in the field plot, this suggests that root growth alone cannot account for the lower total N accumulation.

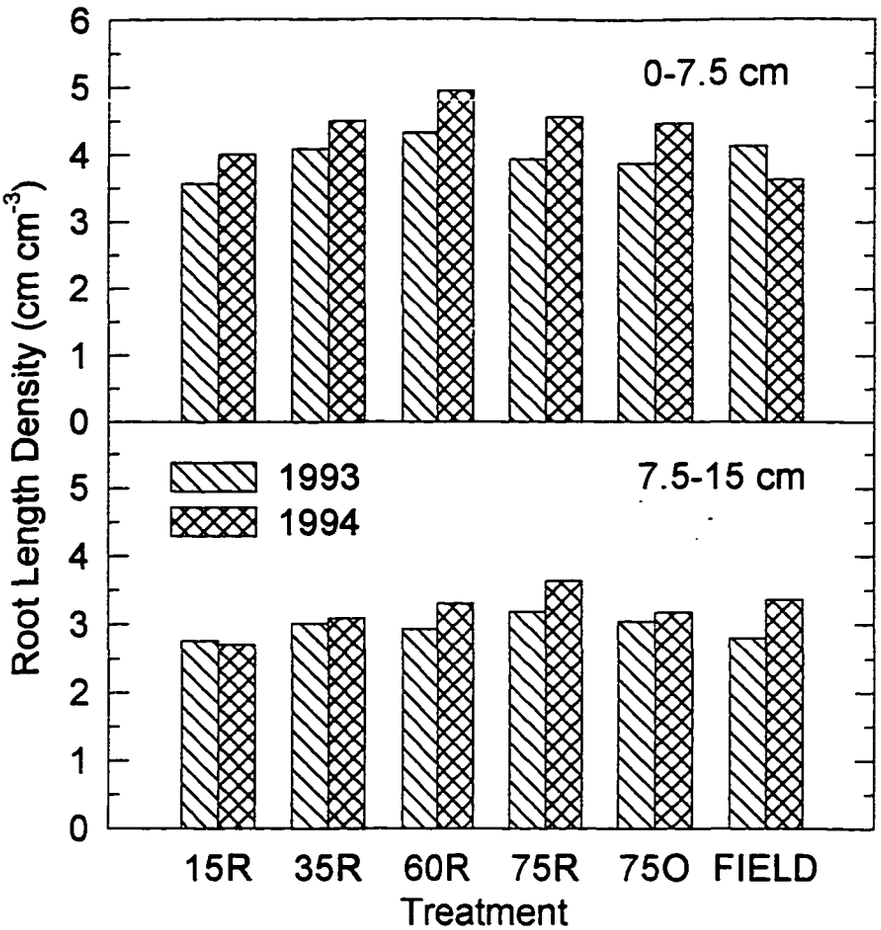

Fig. 5.2. Effect of microplot size on rice root length density in surface soil (0-7.5 cm depth), and subsoil (7.5-15 cm depth). Plot treatments are 15, 35, and 60 cm diameter microplots with retainers, 75 cm square microplot with a retainer, 75 cm square microplot without a retainer, and 2.1 x 7.6 m field plot. Means did not differ from the field plot by Dunnett's test (α=.05) within the same year.

Temperature and pH of both floodwater and soil increased between 0800 and 1600 h each day, then decreased overnight (data not shown). This diurnal pattern was reported previously by Trevitt *et al.* (1988), who also showed that pH changes lagged behind temperature changes by about 2-3 hours, but followed the same pattern. In the present study, differences among treatments were greatest at 1600 h, and no significant differences appeared at 0800 or 1200 h that did not appear at 1600 h. Therefore, only data from 1600 h are discussed.

Floodwater temperature in microplots with retainers was lower than in the field plot (Fig. 5.3). Temperature means increased as microplot size diverged from 35 cm at 44 DAS and 60 cm at 70 DAS. Similarly, Trevitt *et al.* (1988) reported that a decrease in temperature accompanied a decrease in microplot size, and attributed that to the shading effect of the microplot walls. However, plants were at an earlier growth stage in their study. At the growth stages in the present study, particularly at 70 DAS, plant growth accounted for more shading than did the retainer walls. Differences in floodwater temperature in microplots with retainers were likely the result of lack of circulation between the warmer late afternoon floodwater in the field and the cooler floodwater in the microplot. Where circulation could occur, as in the 75 cm microplot without a retainer, the floodwater temperature was not different from the field plot (Fig. 5.3). The limited surface area for heat exchange in the 15 cm microplot could explain the higher temperature there.

Soil temperatures were lower than their respective floodwater temperatures in all plots (Fig. 5.3). This is understandable since solar energy absorbed by the

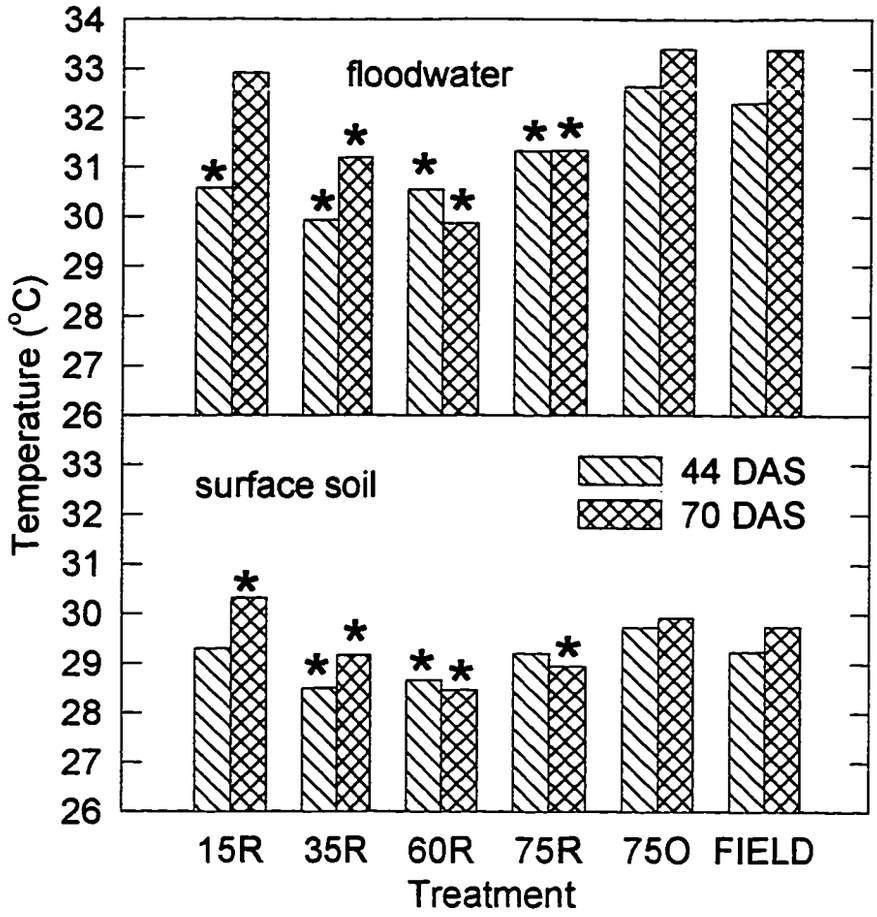

Fig. 5.3. Effect of microplot size on floodwater and surface soil temperature in 1993 (DAS=days after sowing). Plot treatments are 15, 35, and 60 cm diameter microplots with retainers, 75 cm square microplot with a retainer, 75 cm square microplot without a retainer, and 2.1 x 7.6 m field plot. Means that differ from the field plot by Dunnett's test (α=.05) within the same year are marked (*).

floodwater is only partly transferred to the soil. The rest of the energy is retained in the floodwater or dissipated into the environment. The relationship between soil temperature and microplot size followed the same pattern as that for floodwater temperature and microplot size.

At 70 DAS, all enclosed microplots had significantly lower floodwater pH than the field plot (Fig. 5.4). These results are similar to those of Trevitt *et al.* (1988) who found that the lowest floodwater pH occurred in treatments with the lowest temperatures, *i.e.*, the smallest microplots. Differences were not apparent at 44 DAS, except for the 15 cm microplot. It is not likely that the magnitudes of the pH differences would affect plant growth or alter nutrient supply. Soil pH among microplots generally did not differ.

Need for a Retainer

Retainers are used to prevent migration of labeled N. Therefore, microplots without a retainer should show significantly more labeled N outside the microplot than microplots with a retainer. However, labeled-N accumulation by plants harvested outside the microplots was similar in plots with and without retainers (Fig. 5.5). This suggests that the presence of retainers had no effect on the movement of labeled N. Likewise, labeled-N accumulation by plants harvested inside the microplots was similar in plots with and without retainers, regardless of the location of the harvested plants within the microplot (Fig. 5.5). This suggests that there also was no movement of labeled N within a microplot. Labeled-N accumulation by plants inside the microplot was significantly higher than that of plants outside the microplot. In 1993, there was a

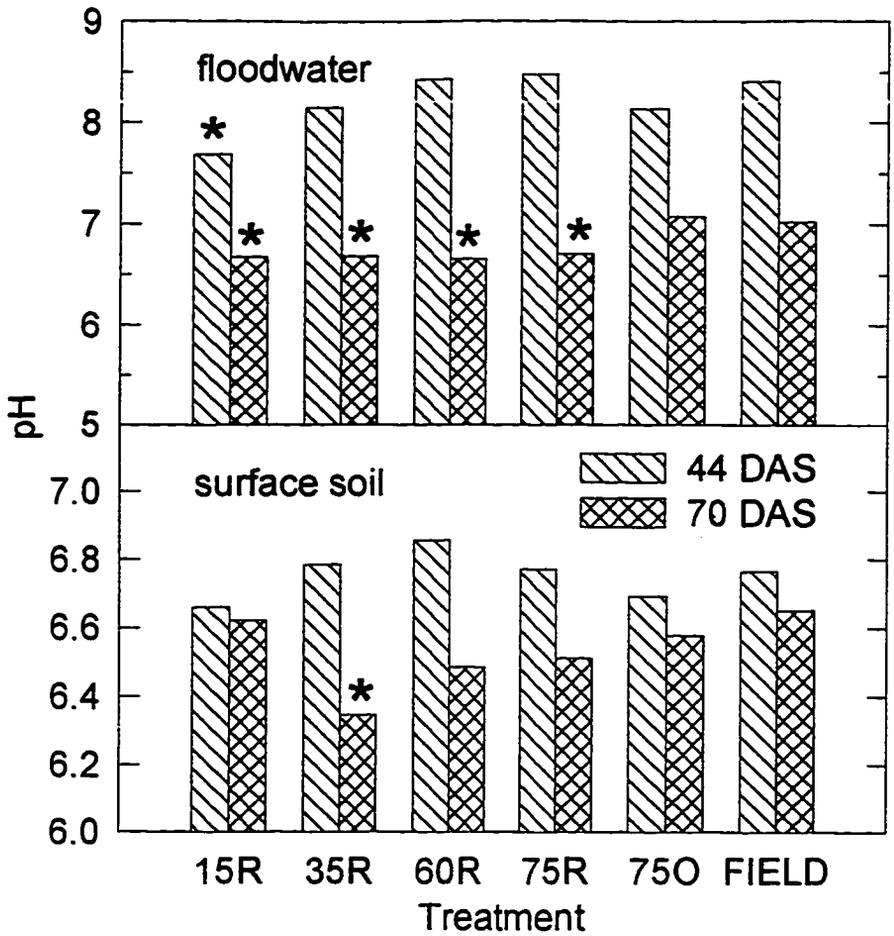

Fig. 5.4. Effect of microplot size on floodwater and surface soil pH in 1993 (DAS=days after sowing). Plot treatments are 15, 35, and 60 cm diameter microplots with retainers, 75 cm square microplot with a retainer, 75 cm square microplot without a retainer, and 2.1 x 7.6 m field plot. Means that differ from the field plot by Dunnett's test (α=.05) within the same year are marked (*).

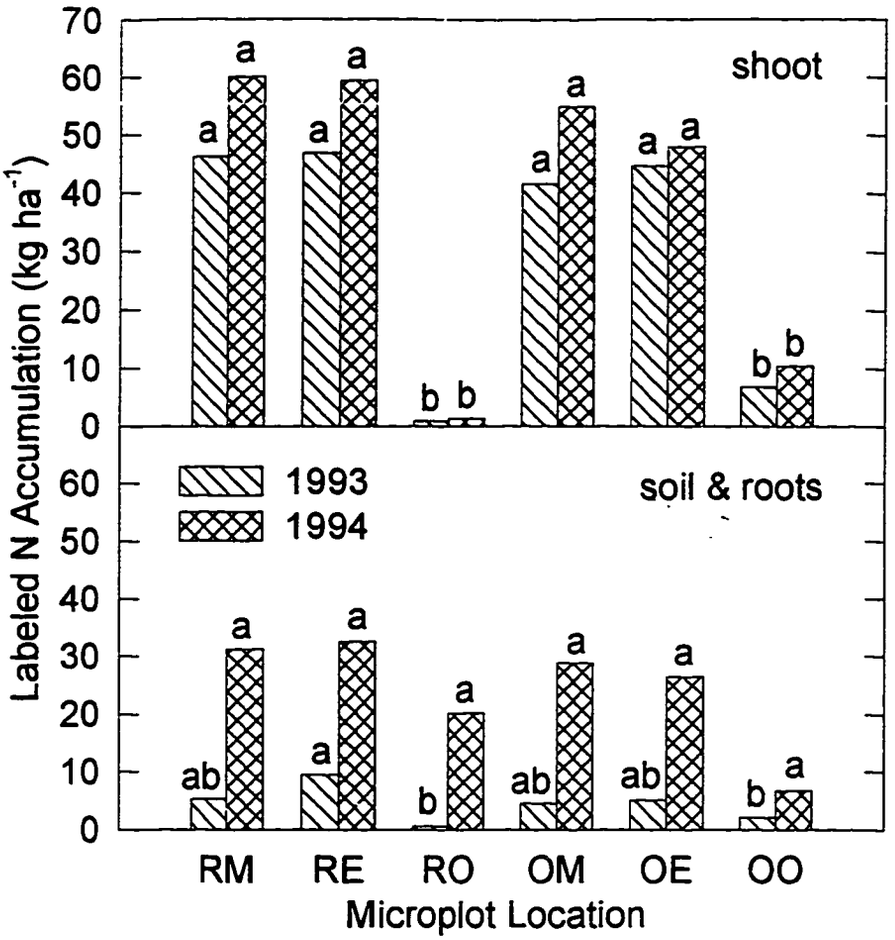

Fig. 5.5. Effect of location within a 75 cm square microplot on labeled N accumulation by rice measured in above ground plant material, and soil and roots. Harvest locations are the middle two rows with a retainer (RM), the edge two rows with a retainer (RE), the outside two rows with a retainer (RO), the middle two rows without a retainer (OM), the edge two rows without a retainer (OE), and the outside two rows without a retainer (OO). Means within the same year with a common letter are not significantly different according to Duncan's Multiple Range test (α=.05).

difference of 34.6 kg labeled N ha^{-1} between the middle and outside of the microplot without a retainer (41.4 *vs.* 6.8 kg ha^{-1}) and in 1994, there was a difference of 37.3 kg labeled N ha^{-1} between the edge and outside of the microplot without a retainer (47.8 *vs.* 10.5 kg ha^{-1}) (Fig. 5.5). Other differences each year between the inside and outside of microplots with or without retainers were even larger. Broadcast N is known to percolate into an unsaturated soil with the floodwater (B.R. Wells, 1994, personal communication), thus minimizing lateral movement. Hence, lateral movement of labeled N was minimal, and a retainer was not necessary.

The largest difference between labeled-N content of plants outside microplots without a retainer and outside microplots with a retainer was 9.2 kg N ha^{-1} (Fig. 5.5). This is a small part of the 151 kg ha^{-1} applied and is agronomically unimportant. Moreover, the presence of labeled-N outside microplots might not be due to migration of labeled N. Roots of plants outside the microplots without retainers were able to grow laterally into areas inside the microplot. Because retainers were inserted 10 cm into the soil, roots of plants outside microplots with retainers would not be able to penetrate the soil down to a 10 cm depth, although they would be able to laterally penetrate the soil below 10 cm. Therefore, plants outside microplots with retainers had less labeled N than plants outside microplots without retainers, but they still contained some labeled N. If retainers were inserted deeper in the soil, even less labeled N should appear in plants outside those microplots.

The presence of residual labeled N in the soil also suggests minimal migration in the floodwater and soil water (Fig. 5.5). Outside the microplots, the largest amount of

labeled N found was 20.1 kg ha^{-1} with a retainer and 6.7 kg ha^{-1} without a retainer in 1994 (Fig. 5.5). These values are small compared to the 151 kg ha^{-1} applied, and again indicate little lateral movement of labeled N. Furthermore, part of the labeled N can be accounted for by roots of plants outside the microplot which took up labeled N inside the microplot. A retainer was not a barrier to N movement, since more labeled N was detected outside the microplot with a retainer than outside the microplot without a retainer.

For studies comparing relative differences between treatments, the difference in N accumulation between microplots with and without retainers should not be of concern. In studies where absolute values of residual soil N or N accumulation must to be made, two options are available. The first is to use a retainer. However, the retainer introduces microclimate modifications such as a decrease in floodwater temperature. The full effect of these microclimate changes is not known, and therefore, can confound experimental results. A second option is not to use a retainer and harvest plants only from the middle of the microplot. The plants at the edge of the microplot would then behave as border rows. This option allows for a buffer between treated and untreated areas, without introducing microclimate modifications.

Substitutes for Field Plots

Microplots can be used as substitutes for field plots in ^{15}N studies if plant growth characteristics and growing conditions do not differ. In this study, the 15 and 35 cm microplots with retainers were not acceptable substitutes for the field plot. These microplots differed from the field plot in many factors measured, particularly

plant dry matter yield for the 35 cm microplot and total N accumulation for the 15 cm microplot (Fig. 5.1).

Both the 60 cm and 75 cm microplots with a retainer were acceptable substitutes for the field plot. The square microplot can be bulk harvested, or the middle plants can be used. Water and soil temperature (Fig. 5.3) and water pH (Fig. 5.4) were different in the microplots compared with the field plot. However, these microclimate differences did not affect plant height (Fig. 5.1), plant dry matter (Figs. 5.1 & 5.6), total N accumulation (Figs. 5.1 & 5.6), or root growth (Fig. 5.2). Plants in the 60 cm microplot were shorter than those in the 75 cm microplot, however dry matter and total N accumulation were not different (Fig. 5.1).

Based on the comparisons of this study, the best substitute for the field plot is a 75 cm microplot without a retainer. The plot can be bulk harvested, or the middle or edge plants can be used. Measured factors in these microplots did not differ from the field plot. Lateral movement of labeled N was insignificant (Fig. 5.5). Furthermore, these plots have the added benefit of a reduced labor requirement because of the absence of retainers.

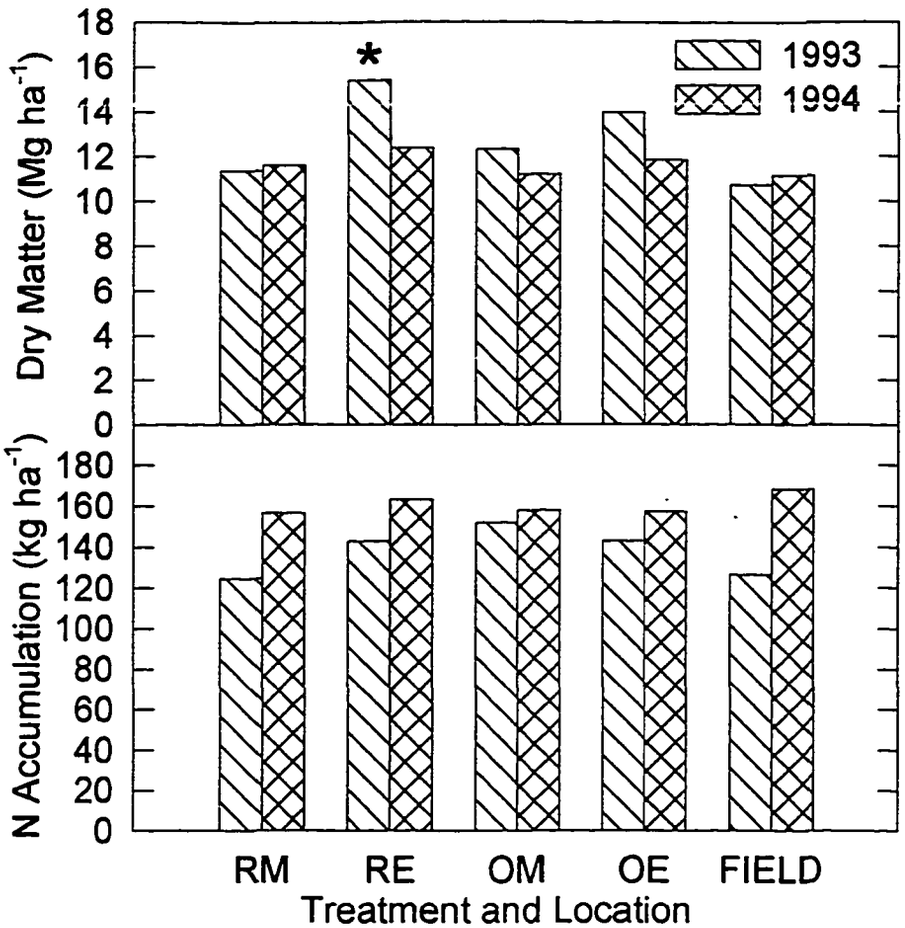

Fig. 5.6. Comparison of dry matter yield and total N accumulation of rice plants harvested from 75 cm square microplots with that of plants harvested from an open field plot. Harvest locations are the middle two rows with a retainer (RM), the edge two rows with a retainer (RE), the middle two rows without a retainer (OM), the edge two rows without a retainer (OE), and a 2.1 x 7.6 m field plot. Means that differ from the field plot by Dunnett's test (α=.05) within the same year are marked (*).

CONCLUSIONS

The purpose of this study was to learn more about N accumulation by rice. This was done through a series of experiments under production-like conditions at the Rice Research Station in Crowley, LA, and in the greenhouse in Baton Rouge, LA.

Nitrogen accumulation was found to occur throughout the growing season, while dry matter increased after panicle differentiation. The late season increase in dry matter was due to ripening grain. While ripening grain accumulated considerable amounts of N, much of this came from translocation from other parts of the plant, rather than an increase in total N accumulation by the plant as a whole. Grain yields were constant from year to year, but straw yield, root growth and N accumulation varied because of air temperature. Very high temperature was related to a decrease in dry matter and N accumulation, and an increase in surface root growth. Dry matter and N accumulation did not differ between drill-seeded and water-seeded culture. When N was applied as a midseason topdress, most topdress N accumulation occurred within 7 days. Dry matter did not fluctuate in response to the midseason topdress.

There was no difference in N accumulation among standard and modern long grain varieties of rice. However, modern medium grain varieties accumulated more N than either. Modern varieties produced more grain than standard varieties, and among modern long grain varieties, semi-dwarf varieties produced more grain than tall varieties. The varieties that produced more grain did so by partitioning more of their accumulated N into grain rather than straw.

Ammonium sulfate and urea were equally effective as sources of N for grain yield. Sulfur, either in ammonium sulfate or added with urea, did not have any effect on N accumulation or grain yield. However, there was more straw at maturity when urea plus S was applied rather than ammonium sulfate.

Microplots used in this study were found to be acceptable substitutes for field conditions. While both the 75 x 75 cm square and the 60 cm diameter microplots each exhibited some differences from the open field, they were minor. However, field labor and microclimate alterations could be minimized with the use of microplots that do not employ retainers, and these were found to best simulate open field conditions.

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VITA

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DOCTORAL EXAMINATION AND DISSERTATION REPORT

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